JOURNAL OF VIROLOGY, May 1986, p. 402–407
0022-538X/86/050402-06$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 58, No. 2

Conserved Regions in Defective Interfering Viral Double-Stranded RNAs from a Yeast Virus

MYUNGHEE LEE, DENNIS F. PIETRAS, MARTIN E. NEMEROFF, BRAM J. CORSTANJE, LOREN J. FIELD,† AND JEREMY A. BRUENN*

Department of Biological Sciences, State University of New York at Buffalo, Buffalo, New York 14260

Received 14 August 1985/Accepted 31 January 1986

We have completely sequenced a defective interfering viral double-stranded RNA (dsRNA) from the *Saccharomyces cerevisiae* virus. This RNA (S14) is a simple internal deletion of its parental dsRNA, M_1, of 1.9 kilobases. The 5′ 964 bases of the M_1 plus strand encode the type 1 killer toxin of the yeast. S14 is 793 base pairs (bp) long, with 253 bp from the 5′ region of its parental plus strand and 540 bp from the 3′ region. All three defective interfering RNAs derived from M_1 that have been characterized so far preserve a large 3′ region, which includes five repeats of a rotationally symmetrical 11-bp consensus sequence. This 11-bp sequence is not present in the 5′ 1 kilobase of the parental RNA or in any of the sequenced regions of unrelated yeast viral dsRNAs, but it is present in the 3′ region of the plus strand of another yeast viral dsRNA, M_2, that encodes the type 2 killer toxin. The 3′ region of 550 bases of the M_1 plus strand, previously only partially sequenced, reveals no large open reading frames. Hence only about half of M_1 appears to have a coding function.

The mycoviruses are double-stranded RNA (dsRNA) viruses with separately encapsidated segments without infectious cycles that persistently infect their host cells. The *Saccharomyces cerevisiae* viruses have one large (L) dsRNA species of about 4.8 kilobases (kb) that encodes the major capsid polypeptide (15) and, in some cases, a second dsRNA (M) of about 1.9 kb that encodes a secreted killer toxin (2) that kills sensitive cells. Suppressive sensitive mutants of the yeast have L and smaller dsRNAs (S) of 0.6 to 1.6 kb that displace M in crosses. These are encapsidated in particles with the same capsid polypeptide (13, 16) and are derived from M by internal deletion, sometimes followed by tandem duplication (4, 6, 10, 16, 31). All preserve the putative transcriptase and replicase recognition sites. There are thus two classes of S dsRNAs, those of 0.6 to 0.8 kb and those, related to this class by tandem duplication, of 1.2 to 1.6 kb. The S dsRNAs displace M by faster replication and are dependent on the same host gene products as M for their maintenance (24). The S dsRNAs have only a portion of the coding region of M and are thus defective in synthesis of toxin. In all respects, then, the S dsRNAs are analogous to defective interfering (DI) RNAs of animal viruses (11, 14, 17, 21, 22).

There are three killer toxin specificities among *S. cerevisiae* virus subtypes. All S dsRNAs characterized so far are derived from the M of subtype 1, M_1. Partial sequence analysis (31) and heteroduplex analysis (10) of one of these, S3, shows that 232 bases are from the 5′ end of the M_1 plus strand and the remainder (about 550 bases) is from the 3′ end of the plus strand. We have cloned cDNA to another S dsRNA (S14) and, in combination with direct RNA sequence analysis, completed its sequence. This is the first yeast viral dsRNA completely sequenced. The breakpoints in S3 and S14 are 21 bp apart at the 5′ end and 10 base pairs (bp) apart at the 3′ end. A third S dsRNA (S733) can be compared with S3 and S14 by analysis of previous T_1 fingerprint data (16). All the S dsRNAs preserve a portion of the 5′ end of the M_1 plus strand (a minimum of 44 bases) and a large portion (up to 550 bases) of the 3′ end of the plus strand. A short (11 base) consensus sequence with twofold rotational symmetry is present five times in this 3′ region. This sequence is not present in the 5′ 1 kb of M_1 (1, 27) or in any of the sequenced regions of L_1 (7, 30; J. A. Bruenn, unpublished data). It may be involved in packaging of M and S, since these dsRNAs require numerous host gene products for their maintenance that are not required by L (34). M_2, the M encoding the type 2 killer toxin, is dependent on the same host gene products as M_1 (32, 35), and this sequence is found in the 3′ portion of the M_2 plus strand as well (12). There are no large open reading frames in either strand of the 3′ 550 bp of M_1.

MATERIALS AND METHODS

Synthesis of viral plus strand. The synthesis of the plus strand by in vitro transcription by viral particles and the purification of the plus strand were as described previously (5).

cDNA synthesis by poly(A) tailing. Synthesis and cloning of cDNA by oligo(dT)-primer synthesis of the first strand on poly(A)-tailed dsRNA was essentially as described previously (8), starting with about 20 μg of tailed RNA and ending with about 200 ng of double-stranded, tailed cDNA.

cDNA synthesis primed by synthetic oligonucleotides. For synthesis of the first strand on the partially purified plus strand, we used 30 μg of crude RNA (10 μg of the plus strand), 0.1 M Tris (pH 8.3 42°C), 10 mM $MgCl_2$, 140 mM KCl, 1 mM deoxynucleoside triphosphates, 20 mM β-mercaptoethanol, 18 ng/μl of 17-base primer, and 200 U of avian myeloblastosis virus reverse transcriptase (Life Sciences, Inc.) in 200 μl at 42°C for 80 min. After Sephadex G-100 chromatography, hydrolysis of RNA in 0.2 N NaOH for 1 h at 50°C, and ethanol precipitation, the second strand was synthesized with 400 ng of the first strand, 0.1 M Tris (pH 7.0), 10 mM $MgCl_2$, 7 mM KCl, 2.5 mM dithiothreitol, 0.5 mM deoxynucleoside triphosphates, 5 ng/μl of primer (26 complementary bases), and 7.2 U of DNA polymerase I Klenow fragment (Bethesda Research Laboratories, Inc.) in 200 μl at 42°C for 1 h. The double-stranded cDNA was isolated by 50% phenol extraction in 0.1% sodium dodecyl

* Corresponding author.
† Present address: Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

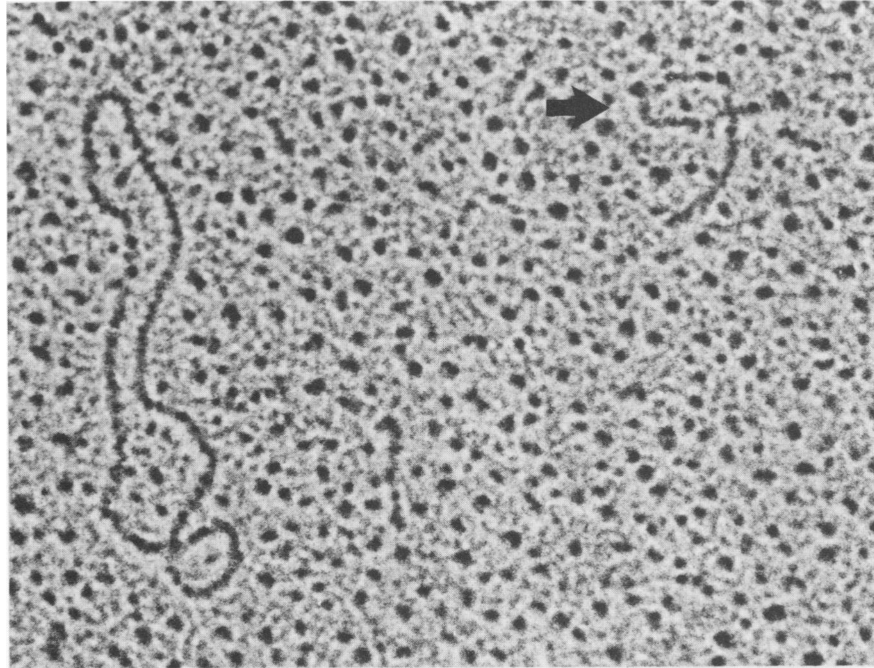

FIG. 1. Heteroduplex analysis of S14. One S14-M_1 heteroduplex (arrow) is shown with an open circular molecule of pBR322 as the internal standard.

sulfate, followed by Sephadex G-100 chromatography and lyophilization.

Southern hybridizations. Southern hybridizations were performed as described previously (18) with nitrocellulose filters (Schleicher & Schuell, Inc.).

Heteroduplex analysis. Heteroduplexing of dsRNAs was performed as described previously (10). Lengths of dsRNAs were calculated as described before (3), with pBR322 open circular DNA as an internal standard.

DNA sequencing. DNA sequence analysis was done by the method of Sanger et al. (25) on cDNA inserts in M13 mp9, with the M13 universal primer (Bethesda Research Laboratories) or various specific synthetic primers. Isotopes were from ICN Pharmaceuticals Inc.

RNA sequencing. Direct RNA sequencing on separated strands of 5′-[^{32}P]cytidine-5′,3′-bisphosphate dsRNAs (19) was performed by the method of Peattie (23). RNA sequencing by the method of Sanger et al. with synthetic oligonucleotides as primers and reverse transcriptase on the in vitro-synthesized viral plus strand was as described previously (28).

Yeast strains. S14 is the suppressive dsRNA present in strain LO14 (from M. Vodkin [33]). S3 or its tandem duplication S1 is the suppressive dsRNA present in strain T132BNK3 (from G. Fink [10, 29]). S733 is the suppressive dsRNA present in strain K733 (from R. Wickner) (16).

RESULTS

Heteroduplex analysis of S14. M_1 and S14 form heteroduplexes with a large deletion loop, in which the double-stranded portion is the length of S14 (Fig. 1). S14, like S3, thus appears to be a simple internal deletion of M_1. S14 has about 230 bp from one end of M_1 and about 530 bp from the other end of M_1 (Fig. 2). Previous sequence analysis has shown that all the S dsRNAs examined (including S14) have the same 3′ termini (at least 20 to 30 bases) as M_1 (5).

Analysis of cDNA clones. Two sets of cDNA clones were made. The substrate for the first-strand synthesis in the first set of experiments was denatured S14 dsRNA tailed in vitro with poly(A). Several hundred cDNA clones resulted from oligo(dT)-primed first-strand synthesis, followed by second-strand synthesis, S1 treatment, oligo (dC) tailing, and insertion into *Pst*I-cut, oligo (dG)-tailed pBR322. Restriction analysis and screening with synthetic oligonucleotides indicated that, for unknown reasons, all of these clones were derived from the 3′ region of the S14 plus strand. Of the cDNA clones, five were partially or completely sequenced by the technique of Sanger et al. after appropriate restriction fragments were subcloned in M13 mp9. One of these extended to the last base of the 3′ end of the S14 plus strand. All the cDNA clones had the same sequence in overlapping regions, indicating that S14 does not have any detectable sequence heterogeneity in this region.

A second set of cDNA clones was constructed for the 5′ region of the S14 plus strand with a synthetic deoxyoligonucleotide of 17 bases complementary to the plus strand within the sequenced region to prime first-strand synthesis. This oligonucleotide was complementary to bases 376 to 392 of the plus strand. The substrate for cDNA synthesis was the in vitro transcript made by *S. cerevisiae* virus ScV-S14 particles. The full-sized first strand (estimated from sequencing

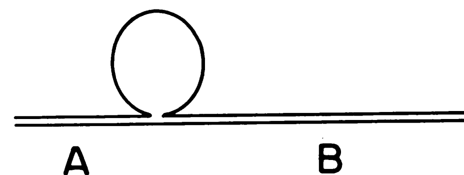

FIG. 2. Statistical analysis of S14-M_1 heteroduplexes. Distance A is 228 ± 35 bp; distance B is 531 ± 45 bp. Distances are the mean values from 14 molecules ± standard deviation.

gels to be 395 bases long) was used as a template for second-strand synthesis with a second synthetic oligonucleotide having the same sequence as bases 14 to 40 of the S14 plus strand. The resultant double-stranded cDNA had the predicted *Bgl*II site present in the homologous region of M_1 cDNA (1, 27) but not the predicted *Pst*I site (data not shown). The cDNA was restricted with *Sau*3A, and fragments were cloned directly into M13 mp9 cut with *Bam*HI. *Taq*I fragments of the cDNA were directly cloned into *Acc*I-cut M13 mp9. Sequencing of these cDNAs, along with direct RNA sequencing from the 3′ end of the minus strand, enabled us to complete the sequence of S14 according to the scheme outlined in Fig. 3. The resultant sequence is presented in Fig. 4.

Major features of the sequence of S14. The breakpoint of S14 is at position 253 of M_1, 21 bp 3′ from the S3 breakpoint (Fig. 4). Reinitiation occurred 540 bases from the 3′ end of the M_1 plus strand in S14 but 550 bases from the 3′ end in S3, since S3 has an additional 10 bp (233 to 243 bp of S3) not present in S14 or in the 5′ region of M_1 (31). In both cases, the conserved region of the M_1 plus strand ends with a C residue and resumes at a C residue. The sequence data would predict heteroduplexes consisting of 540 bp from one end (measured length, 531 ± 45) and 253 bp from the other (measured length, 228 ± 35). The 3′ 540-bp region of S14 (and thus the homologous region of M_1) has no significant open reading frames on either strand.

Reliability of the sequence. The region of S14 derived from the 5′ terminal portion of the M_1 plus strand differs only in one base from the M_1 sequence. This is at position 115, in which S14 has a G and M_1 (1, 27) and S3 (31) each have an A. This destroys the predicted *Pst*I site, which is absent not only in the cloned cDNA from S14 but also in the cDNA prior to cloning (see above). The direct RNA sequence verifies this base difference. If this change were present in the M_1 from which S14 is derived, it would not change the sequence of the preprotoxin (GCG and GCA each code for alanine). The sequence across the second *Sau*3A site at position 165 (used for cloning cDNA fragments) was verified by direct RNA sequencing (Fig. 3). The sequence of bases 209 to 214 of the S14 cDNA is identical to the homologous region of M_1 cDNA (1, 27) but differs from the reported S3 and M_1 sequence (31) determined by direct RNA sequencing (Fig. 4). All of the 5′ region of S14 (1 to 253) has been completed on both strands.

All but the 3′ terminal 31 bp of the 3′ region of S14 has also been sequenced on both strands. This 3′ terminal 31 bp has been sequenced by direct RNA sequencing (6; this work). About 40% of the predicted restriction sites have been checked, including all the 6-base sites. Our sequence from the 3′ region differs from the partial sequence reported for S3 in several positions. Base 265 of S14 is missing in S3. There are no sequence data on M_1 from this region. Bases 470 to 475 at the extreme end of the direct RNA sequencing run from the 3′ end of the plus strand of S3 differ from those of the S14 cDNA. The RNA sequence would predict a restriction site for *Sau*3A which does not exist in the S14 cDNA and would fail to predict a site for *Acc*I which does exist in the cDNA. This is probably not a real sequence difference between S3 and S14, because these changes would alter recognizably the pancreatic products of T_1 oligonucleotides 9 and 16, both of which are present and identical in S14 and S3 (see Table 1). Base 491 is a C in the reported S3 RNA sequence rather than a G as in the S14 cDNA, a change which would obliterate oligonucleotide 17, which is present in both S14 and S3 (Table 1). Base 567 is an A in the M_1 RNA sequence but a G in the S14 cDNA and the S3 RNA sequences, and bases 600 and 601 are the same in S14 and S3 but are reportedly different in M_1. These may be errors in the M_1 sequence, since S3 and S14 arose from different M_1 dsRNAs but are identical in these positions. Finally, base 788 is an A in the S3 sequence but a C in the S14 sequence. This is a genuine strain difference, since this C has been confirmed in S14 by direct RNA sequencing. The published sequencing gel of this region is of the terminal S14 T_1 oligonucleotide (6), and present sequencing results on separated strands of S14 confirm this identification. We have found the same sequence in S2, which was derived from the same M_1 as S14 (33), and we have observed that some M_1s do have an A in this position (V. Brennan and J. Bruenn, unpublished data). Confirmation of a portion of the sequence derived from the 3′ end of the M_1 plus strand was obtained by reverse transcriptase dideoxy sequencing on the S14 plus strand (Fig. 3). There were no differences between the RNA sequence obtained in this way and the sequence of the cloned cDNAs.

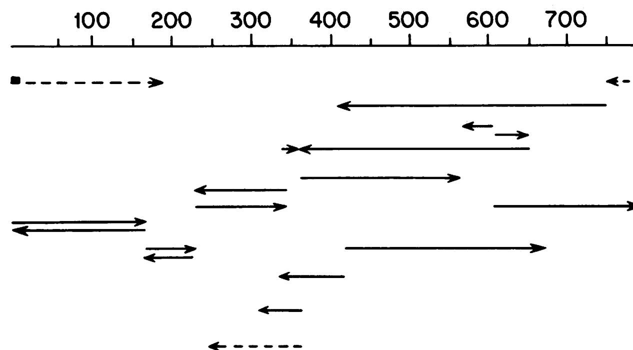

FIG. 3. Strategy for sequencing S14. DNA sequencing runs (5′ to 3′, with arrows at the 3′ ends) are shown by solid lines. RNA sequences are shown by dashed lines. RNA sequences from labeled 3′ ends are indicated by solid boxes at the 3′ ends. Arrows point 3′ to 5′ in these cases. Scale at top is in base pairs.

Confirmation of plus strand. We confirmed the identification of the S14 plus strand deduced from that of M_1 by hybridization of labeled in vitro transcript (plus strand) to M13 subclones of regions of the cDNA from the 3′-terminal portion of the putative S14 plus strand. As expected, the transcript hybridized only to the minus-strand cDNA (Fig. 5). This experiment has been repeated with a *Taq*I fragment from bases 349 to 668 in both orientations in the *Acc*I site of M13 mp9 with the same results (19).

Comparison with other S dsRNAs. We can assign the previously reported large T_1 oligonucleotides of Kane et al. (16) to portions of this sequence by scanning the sequence for predicted large T_1 oligonucleotides. We have matched these predicted T_1 products with the known T_1 oligonucleotides by comparing predicted to determined pancreatic products and predicted to measured mobilities in the two-dimensional gel system used to separate the T_1 oligonucleotides. In this way, we have been able to locate all but one of the previously reported large T_1 oligonucleotides of S14 as well as many of the large T_1 products of M_1. A summary of this analysis is presented in Table 1. All the large M_1 T_1 oligonucleotides from the region deleted in S14 are missing in S14 as well as S3 and S733. The T_1 analysis predicts that S733 should be missing between 86 and 220 bp from the 5′ region of the plus strand of M_1 that is present in S14 and S3. This is consistent with the size of S733 (about 600 bp, or 193

```
                  10        20        30        40        50        60
S14       GAAAAATAAAGAAATGACGAAGCCAACCCAAGTATTAGTTAGATCCGTCAGTATATTATT
S3
M1
                  70        80        90       100       110       120
S14       TTTCATCACATTACTACACCTAGTCGTAGCGCTGAACGATGTGGCCGGTCCTGCGGAAAC
S3                                                              a
M1                                                              a
                 130       140       150       160       170       180
S14       AGCACCAGTGTCATTACTACCTCGTGAAGCGCCGTGGTATGACAAGATCTGGGAAGTAAA
S3
M1
                 190       200       210       220       230       240
S14       AGATTGGCTATTACAGCGTGCCACAGATGGCAATTGGGGCAAGTCGATCACCTGGGGTTC
S3                                   ***   c
M1
                 250       260       270       280       290       300
S14       ATTCGTAGCGAGCCTCACCTTGAGTCTAACTGGTGGCACGCAGCATATCTCACCCTGAGA
S3                                *

                 310       320       330       340       350       360
S14       CTAACTGGCGGCAGGCGACCGGTGAGCATACAGCATGCCCCACTCGATTCGAGACGCGAT
S3

                 370       380       390       400       410       420
S14       TCGCGCTCGTAGGTATCGAGCGGCTACGTTGAGCTATTATGGCAGTGACATGCGATTCGC

                 430       440       450       460       470        480
S14       GCACTGCCAAGATCAGCTCAGCAAAGTTAAGACCAGTATCGGATATGGT*AGACTACTACA
S3                                                         c   tc

                 490       500       510       520       530       540
S14       ATTCGCACAGGTATGAGATTCTCAGTCTAGTGTATGGATGAGTAGTTGAGCCAATGAATC
S3                  c
M1
                 550       560       570       580       590       600
S14       TAGGGTTTAAATTACTATGCATTGACGTATAACAGGTACAAGCGTAGATAATACTTACTA
S3
M1                                  a                               g
                 610       620       630       640       650       660
S14       GGCCCCAGCCGGTACACCCTGTATTGAATAAATACGACTATTTGGCCAGGTCTGGACGGG
S3
M1        a
                 670       680       690       700       710       720
S14       GCAGTCGAATTACTAGGTTGAGCACACACACGTGAATCACACAACATAACAGTGTAGGAA
S3
M1
                 730       740       750       760       770       780
S14       CATAATGTGCCATTCGTAGTCTGAGACGCCGCTAGCCTGGTTTAATGCAACAGCATAGAA
S3
M1
                 790
S14       GAAACACCCATCA
S3               a
M1               a
```

FIG. 4. Sequence of S14. The sequence shown is that of the cDNA, since most of the sequence was performed on cDNA rather than on RNA. This is the plus strand. The plus strand of the RNA begins with a pppG and terminates with an A_{OH}. The region of the sequence from the 5′ end of the M_1 plus strand is in boldface. The boxed sequences are the five repeats of a rotationally symmetrical consensus sequence (see text). Differences among the S14, M_1, and S3 sequences are indicated by lowercase letters (mismatches) and asterisks (deletions). The 5′ M_1 sequence was determined from the cDNA (1, 27), and the S3 and 3′ M_1 sequences were determined by direct RNA sequencing (31). The regions of S14 that have been sequenced in M_1 are bases 1 to 253 and 503 to 793. The regions in S14 that have been sequenced in S3 are 1 to 232, 253 to 327, and 468 to 793.

bp smaller than S14). Remarkably, although these three DI RNAs preserve only a small region of the 5′ end of the M_1 plus strand, they retain a very large region from the 3′ end of the M_1 plus strand: 540 bases in S14, 550 bases in S3, and at least 333 bases in S733. This region has no extensive open reading frames in either the plus strand or the minus strand. Its preservation in every DI RNA suggests that it is important for maintenance of M_1-derived dsRNAs. A survey of this sequence for repeats and inverted repeats found eight repeats of an 11-base consensus sequence, (U/C)GCG AUUCGC(A/G). Five of these, showing at least four of five possible identities in a rotational transformation in the dsRNA, are boxed in the S14 cDNA sequence (Fig. 4).

DISCUSSION

S14 is the first dsRNA from a yeast virus entirely sequenced. The sequence heterogeneity previously observed (6) in T_1 oligonucleotides from the 3′ end of M_1 and S plus strands is not reflected in the cDNA clones from this region nor is it manifest in the direct RNA sequence on separated strands of S14. We believe that this apparent heterogeneity was the result of incomplete T_1 digestion. In contrast, our cDNA cloning has shown that the 3′ end heterogeneity of L (6) is due to the presence of at least two dsRNA species of the same size (9).

There are no obvious sequence similarities at the deletion endpoints of S3 and S14 except for the terminal C and reinitiating C. The viral transcriptase normally initiates with a G and terminates with a penultimate template-encoded C (the terminal A_{OH} or G_{OH} is not template encoded). The replicase does likewise, judging from the terminal sequences of the mature viral dsRNAs (3, 5, 6). Since both transcriptase and replicase are conservative (19, 20, 26), it seems more likely that the transcriptase is responsible for generation of DI RNAs. If the replicase were responsible, the resultant dsRNA would have a very large unpaired single-stranded region (as in the heteroduplex of Fig. 1) which might interfere with its packaging. The deleted region of M_1 is similar in all cases and includes the AU-rich bubble of about 200 bp (10). The 3′ conserved region begins very close to the end of the bubble: the 200-bp AU-rich region begins 1.1 kb from the 5′ end of the plus strand (27), while 550 bp from the 3′ end is not enough to reach this region and M_1 is only 1.8 to 1.9 kb in length. Since the 3′ conserved region does not have any significant open reading frames (on either strand) yet is highly conserved, it may contain sequences recognized by proteins whose functions are essential for viral propagation.

Only a small portion (44 to 82 bp) of the 5′ region is common to all the DI dsRNAs, while a very large region from the 3′ end is common. This 3′ region includes five repeats of a rotationally symmetrical consensus sequence, (U/C)GCGAUUCGC(A/G), which is not present in the 5′ region of M_1 (1, 27) or in any of the sequenced regions of L_1 (7, 30; J. Bruenn, unpublished data). This suggests that this

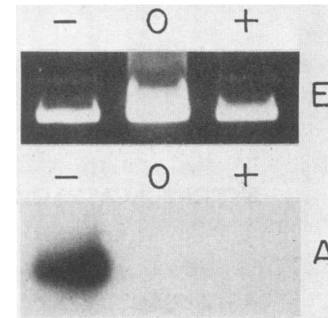

FIG. 5. Identification of the plus strand. The labeled viral plus strand, synthesized in vitro by the viral transcriptase, was hybridized by the Southern method to S14 cDNAs in M13 mp9 from base 340 to 434 (minus [−] strand in phage) or from 430 to 793 (plus [+] strand in phage). M13 mp9 phage DNA without a cDNA insert (0) was a control. E, Ethidium bromide-stained gel; A, autoradiograph of Southern method.

TABLE 1. Large T_1 oligonucleotides of S and M_1

Region (bp)[a]		Spot no. of T_1 oligonucleotide of [b]:		Presence of T_1 oligonucleotide in[c]:			
S14	M_1	S	M_1	S14	S3 (S1)	S733	M_1
1–17	1–17	5	43	+	+	+	+
29–44	29–44	43	25	+	+	+	+
52–83	52–83	1	1	+	+	−	+
131–144	131–144	10	30	+	+	−	+
169–187	169–187	7	6	+	+	−	+
	395–405		4	−	−	−	+
	614–634		21	−	−	−	+
	677–698		5	−	−	−	+
	818–838		4	−	−	−	+
	856–875		6	−	−	−	+
	894–924		1	−	−	−	+
284–297		16	33	+	+	+	+
460–472		16	33	+	+	+	+
472–485		9	55	+	+	+	+
488–500		17	34	+	+	+	+
507–530		2	3	+	+	+	+
540–554		55	30	+	+	+	+
546–559		12	52	+	+	+	+
588–601		45	31	+	+	+	+
619–634		11	27	+	+	+	+
695–712		6	9	+	+	+	+
774–784		19	51	+	+	+	+
782–793		13	13	+	+	+	+

[a] The T_1 oligonucleotides may originate from either the plus or the minus strand in the region identified by base-pair numbers from the plus strand.

[b] The spot numbers are from S1(a) and M_1 as determined by Kane et al. (16). Oligonucleotides were identified with sequence by comparison of predicted and determined pancreatic RNase products (16; Bruenn and Kane, unpublished data). Molarities of the pancreatic RNase products were accurate within experimental error. Some spots contained two large T_1 oligonucleotides of similar mobility (spots 1, 4, 6, 30, and 33 of M_1 and spot 16 of S). Spot 17 of S was originally reported to be absent in S14, but it is present (see the S14 fingerprint in 16; Bruenn and Kane, unpublished data).

[c] +, T_1 oligonucleotide present; −, T_1 oligonucleotide absent.

TABLE 2. Appearance of the consensus sequence (U/C)GCGAUUCGC(A/G) in S14 (M_1) and M_2

RNA	Position from indicated end (bases)		Sequence[a]	% Match
	5′	3′		
S14	343–353	440–450	CUCGAUUCGAG	82
S14	355–365	428–438	CGCGAUUCGCG	100
S14	411–421	372–382	UGCGAUUCGCG	100
S14	494–504	289–299	UGAGAUUCUCA	82
S14	691–701	92–102	CGUGAAUCACA	73
M_2		142–152	UGCGUUUCGCA	91
M_2		49–59	CGCUCUUAGCG	73

[a] Only those sequences with at least four of the five possible identities in a rotational transformation (in the dsRNA) are included.

region may be important for recognition by proteins required for M_1 maintenance but not for L_1 maintenance. There are many such host factors encoded by the MAK genes (34). The M dsRNA of *S. cerevisiae* virus subtype 2 (M_2) is also dependent on these same gene products (32, 35), so we might expect M_2 to have repeats of this same sequence in the 3′ region of its plus strand. Table 2 shows that this sequence is present twice in the 209 bases from the 3′ region of the M_2 plus strand that have been determined (12) and summarizes the fidelity with which the consensus sequence appears in each repeat.

ACKNOWLEDGMENTS

We thank K. Bostian and F. Sherman for synthetic oligonucleotides; M. Vodkin, G. Fink, and R. Wickner for yeast strains; T. H. Chang for advice on direct RNA sequencing; and B. Keitz and D. Castanza for technical assistance.

This work was supported by a Public Health Service grant from the National Institutes of Health (grant GM22200).

LITERATURE CITED

1. **Bostian, K. A., Q. Elliott, H. Bussey, V. Burn, A. Smith, and D. J. Tipper.** 1984. Sequence of the preprotoxin dsRNA gene of type 1 killer yeast: multiple processing events produce a two-component toxin. Cell **36:**741–751.
2. **Bostian, K. A., J. E. Hopper, D. T. Rogers, and D. J. Tipper.** 1980. Translational analysis of the killer-associated virus-like particle dsRNA genome of S. cerevisiae: M dsRNA encodes toxin. Cell **19:**403–414.
3. **Brennan, V. E., L. J. Field, P. Cizdziel, and J. A. Bruenn.** 1981. Sequences at the 3′ ends of yeast viral dsRNAs: proposed transcriptase and replicase initiation sites. Nucleic Acids Res. **9:**4007–4021.
4. **Bruenn, J., and W. Kane.** 1978. Relatedness of the double-stranded RNAs present in yeast virus-like particles. J. Virol. **26:**762–772.
5. **Bruenn, J. A., L. Bobek, V. E. Brennan, and W. A. Held.** 1980. Yeast viral RNA polymerase is a transcriptase. Nucleic Acids Res. **8:**2985–2997.
6. **Bruenn, J. A., and V. E. Brennan.** 1980. Yeast viral double-stranded RNAs have heterogeneous 3′ termini. Cell **19:**923–933.
7. **Bruenn, J. A., K. Madura, A. J. Siegel, Z. A. Miner, and M. Lee.** 1985. Long internal inverted repeat in a yeast viral dsRNA. Nucleic Acids Res. **13:**1575–1591.
8. **Cashdollar, L. W., J. Esparza, G. R. Hudson, R. Chmelo, P. W. K. Lee, and W. K. Joklik.** 1982. Cloning the double-stranded RNA genes of reovirus: sequence of the cloned S2 gene. Proc. Natl. Acad. Sci. USA **79:**7644–7648.
9. **Field, L. J., L. A. Bobek, V. E. Brennan, J. D. Reilly, and J. A. Bruenn.** 1982. There are at least two yeast viral dsRNAs of the same size: an explanation for viral exclusion. Cell **31:**193–200.
10. **Fried, H. M., and G. R. Fink.** 1978. Electron microscopic heteroduplex analysis of "killer" double-stranded RNA species from yeast. Proc. Natl. Acad. Sci. USA **75:**4224–4228.
11. **Guild, G., and V. Stollar.** 1977. Defective interfering particles of Sindbis virus. V. Sequence relationships between SV_{SPD} 42S RNA and intracellular defective viral RNAs. Virology **77:**175–188.
12. **Hannig, E. M., and M. J. Leibowitz.** 1985. Structure and expression of the M_2 genomic segment of a type 2 killer virus of yeast. Nucleic Acids Res. **13:**4379–4400.
13. **Harris, M. S.** 1978. Virus-like particles and double-stranded RNA from killer and non-killer strains of *Saccharomyces cerevisiae*. Microbios **21:**161–176.
14. **Holland, J., E. Grabau, C. Jones, and B. Semler.** 1979. Evolution of multiple genomic mutations during long-term persistent infection by vesicular stomatitis virus. Cell **16:**495–504.
15. **Hopper, J. E., K. A. Bostian, L. B. Rowe, and D. J. Tipper.** 1977. Translation of the L-species dsRNA genome of the killer-associated virus-like particles of *Saccharomyces cerevisiae*. J. Biol. Chem. **252:**9010–9017.
16. **Kane, W. P., D. F. Pietras, and J. A. Bruenn.** 1979. Evolution of defective-interfering double-stranded RNAs of the yeast killer virus. J. Virol. **32:**692–696.
17. **Kennedy, S. I. T.** 1976. Sequence relationships between the genome and the intracellular RNA species of standard and defective interfering Semliki forest virus. J. Mol. Biol. **108:**491–511.
18. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 382–389. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Nemeroff, M. E., and J. A. Bruenn.** 1986. Conservative replication and transcription of *Saccharomyces cerevisiae* viral double-stranded RNA in vitro. J. Virol. **57:**754–758.
20. **Newman, A. M., S. G. Elliott, C. S. McLaughlin, P. A. Sutherland, and R. C. Warner.** 1981. Replication of double-stranded RNA of the virus-like particles in *Saccharomyces cerevisiae*. J. Virol. **38:**263–271.
21. **Nomoto, A., A. Jacobson, Y. Lee, J. Dunn, and E. Wimmer.** 1979. Defective interfering particles of polio virus. Mapping of the deletion and evidence that the deletions in the genomes of DI (I), (II), and (III) are located in the same region. J. Mol. Biol. **128:**179–196.
22. **O'Hara, P., S. Nichol, F. Horodyski, and J. Holland.** 1984. Vesicular stomatitis virus defective interfering particles can contain extensive genomic sequence rearrangements and base substitutions. Cell **36:**915–924.
23. **Peattie, D. A.** 1979. Direct chemical method for sequencing RNA. Proc. Natl. Acad. Sci. USA **76:**1760–1764.
24. **Ridley, S. P., and R. B. Wickner.** 1983. Defective interference in the killer system of *Saccharomyces cerevisiae*. J. Virol. **45:**800–812.
25. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. Sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
26. **Sclafani, R. A., and W. L. Fangman.** 1984. Conservative replication of double-stranded RNA in *Saccharomyces cerevisiae* by displacement of progeny single strands. Mol. Cell. Biol. **4:**1618–1626.
27. **Skipper, N., D. Y. Thomas, and P. Lau.** 1984. Cloning and sequencing of the preprotoxin-coding region of the yeast M1 double-stranded RNA. EMBO J. **3:**107–111.
28. **Smith, A. J. H.** 1980. DNA sequence analysis by primed synthesis. Methods Enzymol. **65:**560–580.
29. **Sweeny, T. K., A. Tate, and G. Fink.** 1976. A study of the transmission and structure of dsRNAs associated with the killer phenomenon in *Saccharomyces cerevisiae*. Genetics **84:**27–42.
30. **Thiele, D. J., E. M. Hannig, and M. J. Leibowitz.** 1984. Multiple L double-stranded RNA species of *Saccharomyces cerevisiae*: evidence for separate encapsidation. Mol. Cell. Biol. **4:**92–100.
31. **Thiele, D. J., E. M. Hannig, and M. J. Leibowitz.** 1984. Genome structure and expression of a defective interfering mutant of the killer virus of yeast. Virology **137:**20–31.
32. **Tyagi, A., R. B. Wickner, C. W. Tabor, and H. Tabor.** 1984. Specificity of polyamine requirements for the replication and maintenance of different double-stranded RNA plasmids in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **81:**1149–1153.
33. **Vodkin, M.** 1977. Induction of yeast killer factor mutations. J. Bacteriol. **132:**346–348.
34. **Wickner, R. B.** 1983. Killer systems in *Saccharomyces cerevisiae*: three distinct modes of exclusion of M_2 double-stranded RNA by three species of double-stranded RNA, M_1, L-A-E, and L-A-HN. Mol. Cell. Biol. **3:**654–661.
35. **Wickner, R. B.** 1983. Genetic control of replication of the double-stranded RNA segments of the killer systems in *Saccharomyces cerevisiae*. Arch. Biochem. Biophys. **222:**1–11.