Volume 9 Number 16 1981 Nucleic Acids Research

# Sequences at the 3' ends of yeast viral dsRNAs: proposed transcriptase and replicase initiation sites

Victoria E.Brennan, Loren Field, Paul Cizdziel and Jeremy A.Bruenn

Division of Cell and Molecular Biology, State University of New York at Buffalo, Buffalo, NY 14260, USA

Received 3 April 1981

## ABSTRACT

ScV is a double-stranded RNA virus of yeast consisting of two separately encapsidated dsRNAs (L and M). ScV-1 and ScV-2 are two dsRNA viruses present in two different yeast killer strains, K1 and K2. Our 3' end sequence analysis shows that the two sets of viral dsRNAs from ScV-1 and ScV-2 are very similar. Consensus sequences for transcriptase and replicase initiation are proposed. A stem and loop structure with a 3' terminal AUGC sequence, like that of several plant virus plus strand RNAs, is present at the putative replicase initiation site of one of the yeast viral RNA plus strands.

## INTRODUCTION

ScV (Saccharomyces cerevisiae virus) is a simple dsRNA (double-stranded RNA) virus with two, separately encapsidated dsRNAs (1,2). Like fungal virus particles (3), ScV particles are communicated from cell to cell only by mating. The larger viral dsRNA (L) encodes the major capsid protein: in vitro translation of denatured L results in the synthesis of the capsid protein (4). The smaller (M) encodes a secreted protein toxin lethal to sensitive strains and also confers resistance to this toxin (5,6). In vitro translation of M1 produces a 32,000 dalton polypeptide which contains the 12,000 dalton toxin protein of ScV1 (7). ScV-M particles are dependent on ScV-L particles for the synthesis of their capsid polypeptide (8,9). Internal deletions of M result in defective-interfering particles containing fragments of M (ScV-S particles; 10,11,12). ScV-M and ScV-S particles are dependent for their replication on products of the nuclear genome that are not necessary for replication of ScV-L particles (13,14).

Thirteen types of yeast killer strains have been described with distinct toxin and resistance functions (15). Two of these, K1 and K2, have been studied in detail (16,17). K1 and K2 strains are able to kill

each other (15) and K1 killer particles exclude K2 killer particles in the same strain: that is, when a K1 haploid is crossed with a K2 haploid the resulting diploids have the K1 killer specificity (18).

Both ScV-L1 and ScV-M1 particles possess a transcriptase activity (19,20,21). The in vitro products of these transcriptases are single-stranded RNAs equal in size to denatured L1 and M1 respectively, and these ssRNAs are extruded from intact particles (19,20,21,22). Hybridization and in vitro translation experiments show that the transcript synthesized by ScV-L1 particles is complementary to only one strand of the double-stranded template and codes for the 88,000 dalton capsid protein of ScV-L1 and ScV-M1 (22). Herring and Bevan (23) have also described a dsRNA polymerase activity possibly associated with replication intermediates.

Both 5' ends of M1 and L1 dsRNAs are pppGp and both 3' termini are $CA_{OH}$ suggesting the end structure Gppp $CA_{OH}$ (24,25). Replication of the yeast dsRNAs would therefore terminate by post-transcriptional addition of a 3' terminal adenosine, as found in the replication of the E. coli RNA bacteriophages (26). L1 and M1 have little sequence homology (10). Sequence analysis of L1 and M1 (25) has shown that both dsRNAs possess both a U-rich and a C-rich 3' terminus. Both 3' ends of M1 and L1 are heterogeneous; the U-rich ends show less heterogeneity than the C-rich ends. There are two recent reviews of the Saccharomyces cerevisiae virus (27,28).

In this report, we compare the sequences of the dsRNAs of ScV1 and ScV2 by 3' end sequence analysis and propose consensus sequences for transcriptase and replicase initiation sites.

## MATERIALS AND METHODS

### Preparation of dsRNA for terminal labeling

The viral RNAs for 3' end labeling were prepared as described previously (25). The cells from which the RNAs were obtained were cloned prior to each preparation.

L1 dsRNA was obtained from non-killer strain S7 (29), the suppressive sensitive strain L02 (30) and the K1 killer strain T158D-SK (31). M1 dsRNA was isolated from the K1 killer strain T158D-SK. L2 and M2 dsRNAs were isolated from the K2 killer strain 1384 (17). L isolated from three different K1 strains (25, Brennan, unpub.), L from five

suppressive strains derived from different K1 strains (25), and L from two laboratory Saccharomyces cerevisiae strains lacking M (25) all have the same 3' ends. We call these dsRNAs L1. Only the L from the K2 killer strain 1384 has different 3' end sequences. We call this L dsRNA L2. M1 from three different K1 strains and S from 5 suppressive strains independently derived from K1 strains have the same 3' ends (25, Brennan, unpub.).

3' End labeling of RNA

dsRNAs were 3' end labeled with 5'-$^{32}$P-cytidine-5'-3'-diphosphate using T4 RNA ligase (PL Biochemicals) as described previously (25). The specific activity of the $^{32}$pCp was between 3000 and 4200 Ci/mmole.

Isolation of the 3' termini

The labeled 3' termini were isolated by partial or complete digestion of the labeled dsRNAs with T1 ribonuclease (Sankyo) followed by separation of the 3' terminal oligonucleotides by the 2D polyacrylamide gel system of DeWachter and Fiers (32). Partial digestion with T1 was accomplished by incubating the end labeled dsRNAs at 37°C for 10 to 15 minutes in a 10 to 20 microliter reaction mixture containing 10 mM Tris-Cℓ pH 7.5, 1 mM EDTA and T1 RNase ($10^{-2}$ to $10^{-4}$ µg/µg RNA). For complete digestion with T1 RNase, the concentration was increased to 5 x $10^{-2}$ µg/µg RNA and the incubation extended to 1 hour at 37°C.

Analysis of end labeled RNAs

For complete hydrolysis with NaOH, RNA samples were dissolved in 5 microliters of 0.5 N NaOH and incubated at 37° for 20 to 24 hours. The samples were run on Whatman 540 paper (pH 3.5) at 5000 volts for 1 hour (33). The T1 ribonuclease products were sequenced by the chemical method of Peattie (34). Throughout, we refer to 3' terminal sequences of the unmodified RNAs and T1 oligonucleotides without their added pCp and with the 5' Gp cleaved by T1.

Electron Microscopy

dsRNA was isolated as described by Bruenn and Kane (10). Spreading was basically as described by Fried and Fink (11), but onto 0% formamide, 8.16 mM Tris-Cl, 0.816 mM EDTA (pH 8.5). All micrographs were taken on a Hitachi HU500 at 10,000 x and enlarged 7 x on a Simmons-Omega enlarger. Contour lengths were measured with a Numonics graphic calculator. pBR322 open circular DNA (4362 bp.; 35) was included as an internal standard. The lengths of dsRNA in base pairs were calculated using a dsDNA/dsRNA ratio of 3.4/3.0 (27). M1 was from T158DSK (31) or 1406 (36).

## RESULTS

### Lengths of dsRNAs

By comparison with pBR322 open circular DNA (see Materials and Methods), we find that L1 is 4804 ± 240 bp (N=140) and L2 is 4926 ± 167 bp (N=47). Since we have no control dsRNA of known sequence, these are approximations, but they are similar to previous estimates of the length of L1 (27) and are consistent with our inability to separate L1 and L2 by gel electrophoresis (not shown). Similarly, M1 is 1965 ± 104 bp (N=91) and M2 is 1804 ± 88 bp (N=109). These results are consistent with the fact that M1 is larger than M2 by gel electrophoresis (17, Field, unpub. results).

### Isolation of labeled 3' termini of viral dsRNAs

The viral RNAs were labeled at their 3' ends with $^{32}$pCp by T4 RNA ligase. The incorporation of label was between 10 and 40% of the theoretical yield. Separation of the labeled 3' termini of the double-stranded RNA was achieved by partial or complete T1 digestion followed by 2D polyacrylamide gel electrophoresis (32).

The only labeled product of complete alkaline hydrolysis of all the isolated 3' termini from L1, L2, M1 and M2 was Ap. This is in agreement with previous results (25), which we interpret as indicating that the synthesis of yeast dsRNAs terminates by post-transcriptional addition of 3' terminal adenosine.

### Sequence analysis of 3' termini of K1 dsRNAs

We have sequenced isolated 3' labeled termini from complete T1 digestion of L1 and M1 dsRNAs (25) by the chemical method of Peattie (34). In each case there were several 3' labeled T1 oligonucleotides visible in autoradiographs of the 2D gels, representing the two ends of the double-stranded molecules. This indicated that the 3' termini of both L1 and M1 were heterogeneous (25). Complete T1 digestion of L1 gave two major labeled products, a U-rich 3' oligonucleotide (GAAUUUAAAAAUUUUUCA$_{OH}$) and GCA$_{OH}$ (25). In the present study partial T1 digestion of 3' labeled L1 was employed to extend the sequences of the GCA$_{OH}$ and U-rich termini. Several 3' partial T1 oligonucleotides from the 2D gels were recovered and sequenced. Figure 1 shows a sequencing gel of a partial T1 oligonucleotide corresponding to the U-rich end of L1. From this (and other) partial T1 oligonucleotides we derive the 3' end sequence GUAUGGGGAGUUAUAUGAAUUUAAAAAUUUUUCA$_{OH}$. Figure 2 shows a sequencing gel of

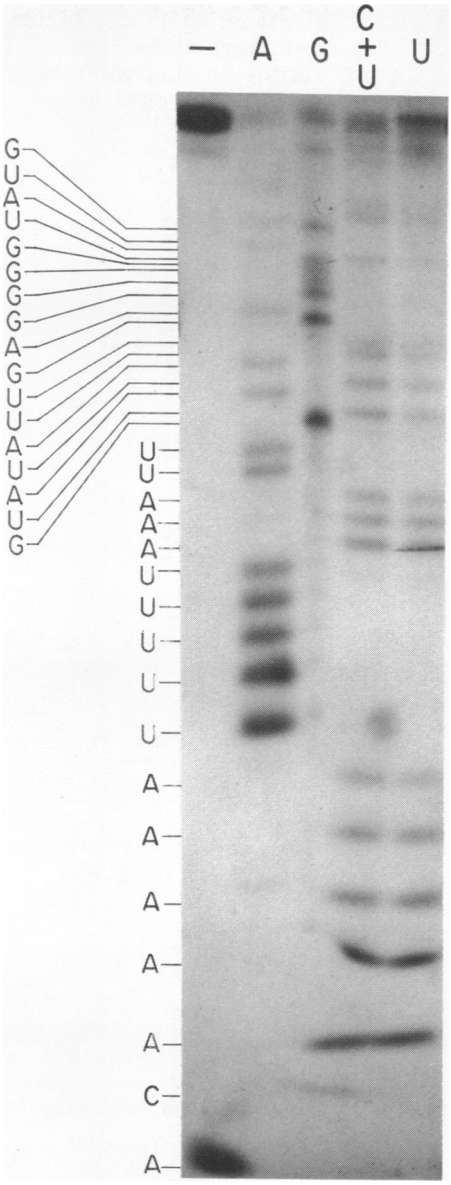


Figure 1. Sequencing gel of a partial T1 oligonucleotide corresponding to the U-rich 3' end of L1. The chemical cleavage products of the L1 3' end labeled, T1 partial oligonucleotide were run on a 20% polyacrylamide, 7 M urea gel (34).

the longest T1 oligonucleotide analyzed corresponding to the $GCA_{OH}$ end of L1. The 3' terminus of this oligonucleotide has the sequence $GCAAAAAGAUAGGGAAUUACCCAUAUGCA_{OH}$. The run of 3 C's not visible in this print has been verified by sequencing several other partial T1 products

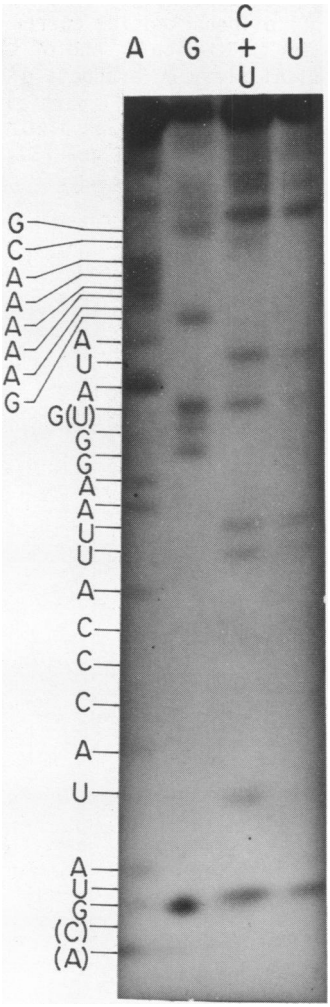


Figure 2. Sequencing gel of a partial T1 oligonucleotide corresponding to the C-rich 3' end of L1. Procedures were as in Fig. 1. The run of 3 C's not visible in this print has been verified by sequencing several other partial T1 products.

(not shown).

In some 2D gels of partial T1 digests of 3' labeled L1, an alternate sequence was found which is believed to be derived from the U-rich end of L1. This alternate sequence, GUGUGCGAGUGGAAAAAUUCA$_{OH}$, corresponds to a minor, complete T1 digestion product of 3' labeled L1, namely GAAAAAUUCA$_{OH}$ (25).

The 3' termini of M1 dsRNA have already been described (25). M1 also has a U-rich and a C-rich 3' terminus. Table I and Table II summarize and compare the C-rich and U-rich ends of L1 and M1.

TABLE I. The U-rich 3' ends of yeast viral dsRNAs

| RNA | U-rich 3' end sequence (30 20 10) |
|---|---|
| L1 | GUAUGGGGAGUUAUAUGAAUUUAAAAAUUUUUCA$_{OH}$ |
| L2 | GAGU GUAUGAAUAUUCAAAUUAUUCA$_{OH}$ (G) |
| L alternate | GUGUGCGAGUGGAAAAA UUCA$_{OH}$ |
| M1 | GUUUAUUAUUUCUUUAUUUUUCA$_{OH}$ |
| M2 | GUAGUCUCUUUAUUUUUCA$_{OH}$ (C U) |

Sequence analysis of 3' termini of K2 dsRNAs

Isolated 3' termini from complete and partial T1 digests of L2 and M2 were sequenced. Complete T1 digestion of 3' end labeled L2 results in the U-rich 3' oligonucleotide GAAUAUUCAAAUUAUUCA$_{OH}$ (Figure 3), the U-rich alternate 3' oligonucleotide GAAAAAUUCA$_{OH}$ and GCA$_{OH}$. Partial $T_1$ digestion was used to extend the sequences of both termini. The U-rich terminus was extended to the sequence GAGUGUAUGGAAUAUUCAAAUUAUUCA$_{OH}$. The longest partial $T_1$ oligonucleotide corresponding to the GCA$_{OH}$ end had the sequence GUAAAUAUAAGAGCUUAUACACAUAUGCA$_{OH}$ (Figure 4). The CACA sequence unclear in this print has been verified by other gels. The superscript nucleotides indicate ambiguous bases. These ambiguities are probably not

TABLE II. The C-rich 3' ends of yeast viral dsRNAs

| RNA | C-rich 3' end sequence (30 20 10) |
|---|---|
| L1 | GCAAAAAGAUAGGGAAUUACCCAUAUGCA$_{OH}$ |
| L2 | (G G U) GUAAAUAUAAGAGCUUAUACACAUAUGCA$_{OH}$ |
| L2' | GACAAUAAAAAUAUGCA$_{OH}$ |
| M1 | (G G) (G G U GUUA C) (U UUU U CU CUG UCUCCU U) GCACCCACCAACACCACACAACGAAACACCCAU CA$_{OH}$ |
| M2 | GAAU(U)ACUACAGGUACAUUUACCUAG CA$_{OH}$ |

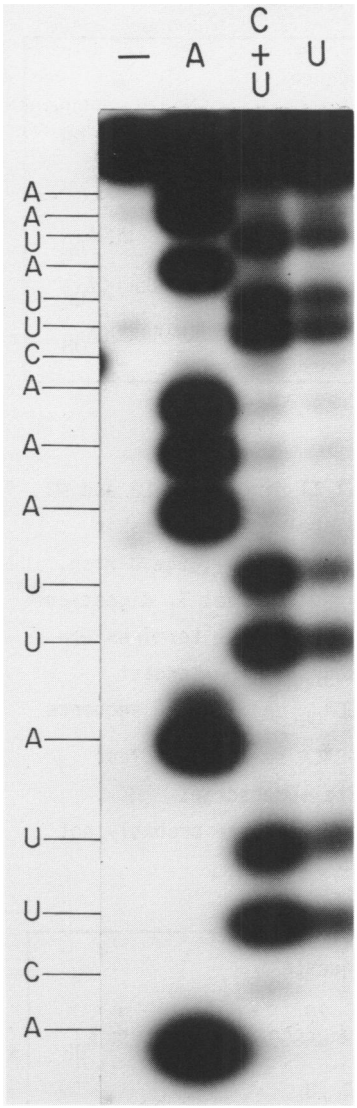


Figure 3. Sequencing gel of a complete T1 digestion oligonucleotide corresponding to the U-rich 3' end of L2. Procedures were as in Fig. 1.

artifacts: the same ambiguities appeared in several independent sequencing experiments. This indicates that the larger 3' terminal $T_1$ oligonucleotides from the C-rich end of L2 are heterogeneous, consisting of mixtures of isomers not separated by the 2D gel electrophoresis system, as previously observed for the M1 C-rich end (25). An alternate C-rich end for L2 (L2') was also found in some partial digests. This had

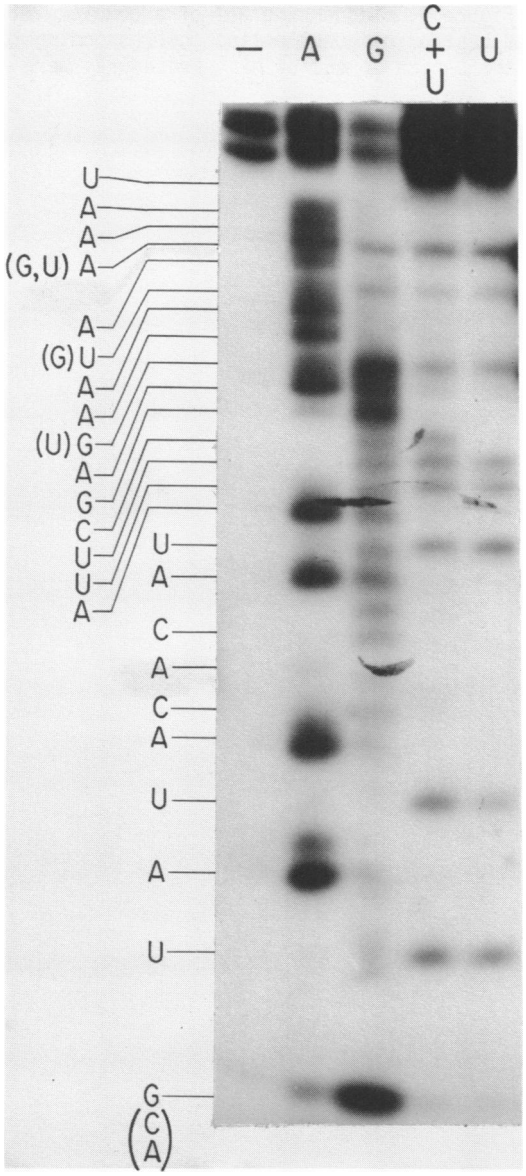


Figure 4. Sequencing gel of a partial T1 oligonucleotide corresponding to the C-rich 3' end of L2. Procedures were as in Fig. 1. The CACA sequence not clear in this gel has been verified by other gels.

the sequence GACAAUAAAAAUAUGCA$_{OH}$ (data not shown). This confirms the heterogeneity of the C-rich 3' end of L2.

Complete $T_1$ digestion of 3' end labeled M2 produces only a U-rich sequence GUCUCUUUCAUUUUUUCA$_{OH}$ (Figure 5)and GCA$_{OH}$. There is not the multiplicity of 3' ends characteristic of M1 (25). By partial $T_1$

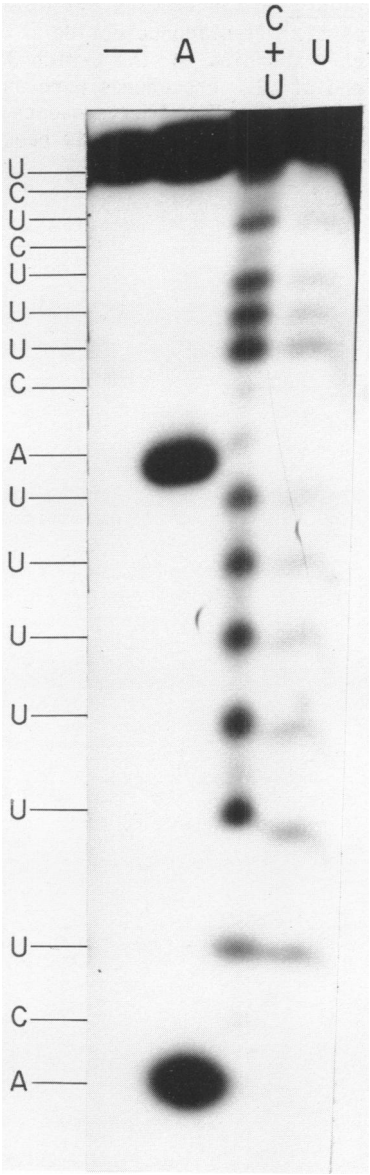


Figure 5. Sequencing gel of a complete T1 digestion oligonucleotide corresponding to the U-rich 3' end of M2. Procedures were as in Fig. 1.

digestion the sequence of the $GCA_{OH}$ terminus was extended to GAAUUACUAC-AGGUACAUUUACCUAGCA$_{OH}$ (Figure 6) and the U-rich sequence to GUAGUCUCUUUCA-UUUUUUCA$_{OH}$. No other 3' end sequences were detected. The band compression observed through the region ACAGG of the C-rich end of M2 (Fig. 6) may be

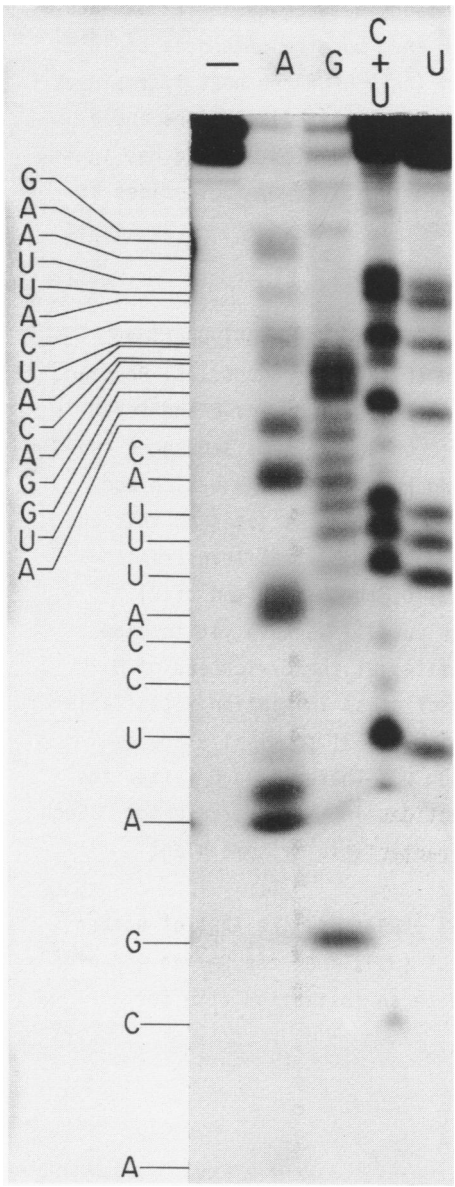


Figure 6. Sequencing gel of a partial T1 oligonucleotide corresponding to the C-rich 3' end of M2. Procedures were as in Fig. 1.

due to the secondary structure postulated in Fig. 8. All of the dsRNAs examined are compared in Tables I and II. L2 differs from L1 by 5 substitutions, a single base deletion and a single base insertion in the first 27 nucleotides at the U-rich end, and by 1 substitution in the first

12 nucleotides at the C-rich end. The homology between L1 and L2 extends at least to 27 nucleotides from the U-rich end, but the sequences begin to diverge at position 12 from the C-rich end (only 8 of the next 17 residues are identical). M2 differs from M1 by two-single base insertions and a substitution in the first 16 nucleotides from the U-rich end and has 10 substitutions and one-one base insertion in the first 27 nucleotides at the C-rich end.

## DISCUSSION

Since ScV-L and ScV-M contain the same major capsid polypeptide, which is at least 98% of the viral protein (D. Reilly, unpub., K. Bostian, personal comm.), the in vitro transcriptase activity of ScV-L and ScV-M is probably the same, and should therefore recognize the same sequences in L and M. The 3' end with the most striking homology is the U-rich end. Consequently, we have speculated that transcription initiates at this end (25). Our recent experiments confirm that in vitro viral transcription initiates at the penultimate nucleotide (C) of the U-rich end of L1 (Brennan, Bobek, and Bruenn, submitted for pub.). We have yet to show directly that in vitro transcription initiates at the U-rich end of M. Presuming that it does, we propose a transcriptase initiation site (Table III) that is a sequence of 17 bases of which the 3' terminal 9 are essentially unambiguous and 16 of 17 are A or U. There is no G (after the 5' pppGp) for at least the first 33 nucleotides in the L1 transcript. Such U-rich 5' ends without G residues are characteristic of some plant virus mRNAs (37).

If the replication of the yeast viral dsRNAs is like that of either the dsRNA viruses of fungi (38) or reovirus (39), then the C-rich end of L and M mRNA transcripts ($\ell$ and m) should be an initiation site for a

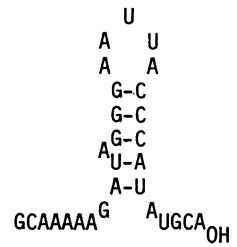


Figure 7. Secondary structure at the C-rich end of $\ell$1 ($\Delta F$ = -5 kcal/mole).

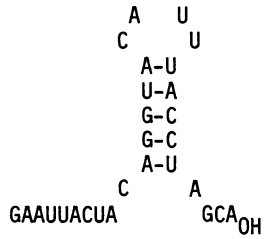


Figure 8. Secondary structure at the C-rich end of m2 ($\Delta F$ = -6.2 kcal/mole)

replicase. The C-rich end of L1 does have a structure very similar to that of the 3' ends of some plant virus genomic (plus strand) RNAs, which serve as recognition sequences for coat protein-replicase complexes (40). In the $\ell 1$ transcript, this is a hairpin and loop structure of $\Delta F$ = -5 kcal/mole (41) followed immediately by the sequence AUGCA$_{OH}$ (Fig. 7). This is precisely the structure (and 3' terminal AUGC) present in several plant virus RNAs (37). The only similarly located loop structure in $\ell 2$ is not thermodynamically stable($\Delta F$ = + 0.4 kcal/mole). A hairpin and loop structure can be formed at the C-rich end of M2, but the 3' terminal AUGC sequence becomes just AGC (Fig. 8). Neither the loop structure nor the 3' terminal AUGC are present at the C-rich end of m1. A consensus replication recognition sequence at the C-rich end can be derived (Table III) if that sequence may be present at a slightly variable distance from the 3' A$_{OH}$.

TABLE III. Consensus sequences

C-rich end consensus sequence (replicase initiation)

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G/C | A/U | A/U | A/U | U | A | C | C | C | A | U | ( | A | U | G | ) | C | A$_{OH}$ | | | | |
| 4 | 4 | 5 | 5 | 4 | 5 | 4 | 3 | 3 | 5 | 4 | ( | 3 | 3 | 3 | ) | 5 | 5 | | | | |

(number that fit at each nucleotide of 5 sequences determined)

U-rich end consensus sequence (transcriptase initiation)

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| U/A | A | U | U/A | U | U/A | U/A/U | A | A | A | U | U | U | U | U | C | A$_{OH}$ | | | | | |
| 4 | 3 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 4 | 4 | 4 | 3 | 5 | 5 | 5 | 5 | | | | | |

(number that fit at each nucleotide of 5 sequences determined)

If both the stem and loop structure and the terminal AUGC sequence are important for accurate replication, the observed dramatic sequence heterogeneity at the 3' end of M1 (25) is thereby explained: m1 has neither stem and loop nor AUGC. M2 is less heterogeneous at this end and has a stem and loop structure in m2. L2 is more heterogeneous at the C-rich end than L1, and ℓ2 has only the AUGC sequence without the nearby stem and loop structure present in ℓ1. The considerable number of sequence differences between these closely related viral dsRNAs (L1 and L2, M1 and M2) is also consistent with the high mutation rate postulated for ScV (25). Replication of M requires products coded by the host genome not required for replication of L (14). These cellular functions may be additional requirements for replication of M mandated by the loss of a 3' end C-rich sequence required by the (putative) ScV capsid protein replicase alone (perhaps the sequence AUGC). The exclusion of ScV-M2 particles in K1 x K2 crosses (17) may also be a consequence of sequence differences at the C-rich end (Field, Brennan, and Bruenn, in preparation).

ACKNOWLEDGEMENTS

We thank C. McLaughlin, M. Vodkin, G. Fink and R. Wickner for strains and the NIH (Grant number GM22200) for support.

REFERENCES

1. Buck, K.W., Lhoas, P. and Street, B.K. (1973) Biochem. Soc. Trans. 1, 1141-1142
2. Herring, A.J. and Bevan, E.A. (1974) J. Gen. Virol. 22, 387-397
3. Saksena, K.N. and Lemke, P.A. (1978) In Comprehensive Virology 12, 103-143, Plenum Press, New York
4. Hopper, J.E., Bostian, K.A., Rowe, L.B. and Tipper, D.J. (1977) J. Biol. Chem. 252, 9010-9017
5. Bevan, E.A. and Makower, M. (1963) In Genetics Today, Geerts, Ed., 1, 202-203, Macmillan, The Hague
6. Palfree, G.E. and Bussey, H. (1979) Eur. J. Biochem. 93, 487-493
7. Bostian, K.A., Hopper, J.E., Rogers, D.T. and Tipper, D.J. (1980) Cell 19, 403-414
8. Herring, A.J. and Bevan, E.A. (1975) In Molecular Biology of Nucleocytoplasmic Relationships, Chap. III, 6, 149-154
9. Harris, M.S. (1978) Microbios. 21, 161-176
10. Bruenn, J. and Kane, W. (1978) J. Virol. 26, 762-772
11. Fried, H.M. and Fink, G. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 4224-4228
12. Kane, W., Pietras, D. and Bruenn, J. (1979) J. Virol. 32, 692-696
13. Somers, J.M. (1973) Genetics 74, 571-579
14. Wickner, R.B. (1978) Genetics 88, 419-425

15. Young, T.W. and Yagiu, M. (1978) Antonie van Leeuwenhoek 44, 59-77
16. Wickner, R.B. (1976) Bacteriol. Rev. 40, 757-773
17. Wickner, R.B. (1980) Cell 21, 217-226
18. Naumov, G.I. and Naumova, T.I. (1973) Genetika 9, 140-145
19. Herring, A.J. and Bevan, E.A. (1977) Nature 268, 464-466
20. Welsh, J.D. and Leibowitz, M.J. (1980) Nucl. Acids Res. 8, 2365-2375; Welsh, J.D., Leibowitz, M.J. and Wickner, R.B. (1980) Nucl. Acids Res. 8, 2349-2363
21. Hastie, N., Brennan, V. and Bruenn, J. (1978) J. Virol. 28, 1002-1005
22. Bruenn, J., Bobek, L., Brennan, V. and Held, W. (1980) Nucl. Acids Res. 8, 2985-2997
23. Bevan, E.A. and Herring, A.J. (1976) In Genetics Biogenesis and Bioenergetics of Mitochondria, Bandlou, W., Schweyen, R.J., Thomas, D.Y., Wolf, K. and Kandewitz, F., Eds., 153-162, Walter de Gruyter
24. Bruenn, J. and Keitz, B. (1976) Nucl. Acids Res. 3, 2427-2436
25. Bruenn, J. and Brennan, V. (1980) Cell 19, 923-933
26. Weber, H. and Weissmann, C. (1970) J. Mol. Biol. 51, 215-224
27. Bruenn, J. (1980) Ann. Rev. Microbiol. 34, 49-68
28. Wickner, R.B. (1979) Plasmid 2, 303-322
29. Oliver, S.G., McCready, S.J., Holm, E., Southerland, R.A., McLaughlin, C.S. and Cox, B.S. (1977) J. Bact., 130, 1303-1309
30. Vodkin, M. (1977) J. Bact. 132, 346-348
31. Sweeney, T.K., Tate, A. and Fink, J.R. (1976) Genetics 84, 27-42
32. DeWachter, R. and Fiers, W. (1972) Anal. Biochem. 49, 184-197
33. Barrell, B.G. (1971) In Procedures in Nucleic Acid Research 2, 751-779, Harper and Row
34. Peattie, D.A. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 1760-1766
35. Sutcliffe, J.G. (1978) Nuc. Acids Res. 5, 2721-2728
36. Toh-E, A., Guerry, P. and Wickner, R.B. (1978) J. Bacter. 136, 1002-1007
37. Koper-Zwarthoff, E.C., Brederode, F.T., Veeneman, G., Van Boom, J.H. and Bol, J.F. (1980) Nucl. Acids Res. 8, 5635-5647
38. Buck, K.W. (1975) Nuc. Acids Res. 2, 1889-1902
39. Silverstein, S.C., Christman, J.K. and Acs, G. (1977) Ann. Rev. Biochem. 45, 375-408
40. Koper-Zwarthoff, E.C. and Bol, J.F. (1980) Nucl. Acids Res. 8, 3307-3318
41. Tinoco, I., Borer, P.N., Dengler, B., Levine, M.D., Uhlenbeck, O.C., Crothers, D.M. and Gralla, J. (1973) Nature New Biol. 246, 40-41