Overlapping Genes in a Yeast Double-Stranded RNA Virus

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The *Saccharomyces cerevisiae* viruses have a large viral double-stranded RNA which encodes the major viral capsid polypeptide. We have previously shown that this RNA (L1) also encodes a putative viral RNA-dependent RNA polymerase (D. F. Pietras, M. E. Diamond, and J. A. Bruenn, *Nucleic Acids Res.*, 16:6226, 1988). The organization and expression of the viral genome is similar to that of the *gag-pol* region of the retroviruses. The complete sequence of L1 demonstrates two large open reading frames on the plus strand which overlap by 129 bases. The first is the gene for the capsid polypeptide, and the second is the gene for the putative RNA polymerase. One of the products of in vitro translation of the denatured viral double-stranded RNA is a polypeptide of the size expected of a capsid-polymerase fusion protein, resulting from a -1 frameshift within the overlapping region. A polypeptide of the size expected for a capsid-polymerase fusion product was found in virions, and it was recognized in Western blots (immunoblots) by antibodies to a synthetic peptide derived from the predicted polymerase sequence.

The *Saccharomyces cerevisiae* viruses (ScV) are a family of double-stranded RNA (dsRNA) viruses present in most laboratory strains of this yeast. Like other fungal viruses, they are noninfectious, segmented dsRNA viruses in which the segments are separately encapsidated. Transmission is by mitosis or by mating, although spheroplasts can be infected by viral particles (16). A large viral dsRNA (L) encodes the major capsid polypeptide (30). A second dsRNA, M, present in some strains, encodes a secreted toxin that kills cells without the virus (3). L and M are separately encapsidated in particles with about 60 monomers (45) or 120 monomers (11) of the major capsid polypeptide P1, which has been estimated to be 75 kilodaltons (kDa; 42), 81 kDa (48), or 88 kDa (30). This is an attractive model system for dsRNA viruses. There is only a single major capsid polypeptide present in the particle; only a single viral dsRNA encodes all the necessary viral functions; the viral enzymatic activities are limited, since the viral RNAs are uncapped (9); transcription and replication are conservative, as in reovirus (22, 40, 55); host-viral interactions are amenable to genetic analysis (53); and there are no cellular DNA copies of the viral genome (26).

We have sequenced the major species of the large viral dsRNA (20) from ScV subtype 1 (L1). The plus strand of 4,580 bases (b) has two large open reading frames that overlap by 129 b. The first encodes the putative major viral capsid polypeptide, with a predicted size of 76 kDa. The second open reading frame could be read by a translational frame shift of -1 within the overlap region, to encode a fusion polypeptide of 171 kDa. Such a polypeptide, which is recognized by antibodies to P1, does exist in ScV particles (45). A product of this size results from translation of the denatured L1 dsRNA in vitro. The predicted polypeptide of the second open reading frame has extensive homologies to the RNA-dependent RNA polymerases (RDRPs) of single-stranded RNA viruses (44; this work). Antibodies raised against a synthetic peptide derived from the predicted sequence of the viral RDRP reacted specifically with a poly-

peptide of the expected size of a capsid-polymerase (*cap-pol*) fusion product.

MATERIALS AND METHODS

Strains. The cDNA clones used for sequencing L1 were derived from the L1 present in strain LO14 (52). The sequence shown is entirely derived from the L1 of LO14. Comparison was made to cDNA clones from the L1 of strain S7 (42), and only minor differences were evident. All the in vitro translations and Western blots (immunoblots) were performed with RNA or particles from both strains, with identical results.

cDNA synthesis and cloning. ds cDNAs were synthesized by using synthetic oligonucleotide primers as previously described (41), except that second-strand synthesis and amplification sometimes utilized the polymerase chain reaction (46). Clones in pUC and M13mp vectors were constructed by blunt-end ligation of undigested cDNA or by integration of *Hae*III, *Sau*3A, *Bgl*II, or *Eco*RI fragments into appropriately cleaved vector DNAs.

DNA and RNA sequencing. DNA sequencing on ds pUC template DNAs or single-stranded M13mp template DNAs was by the technique of Sanger et al. (47). RNA sequencing was of separated strands of L1 labeled with ³²pCp by the chemical method (43). Synthetic deoxyoligonucleotides were made by the SUNY/Buffalo Center for Applied Molecular Biology and Immunology.

In vitro translation. In vitro translation was performed as previously described (28) on purified, denatured L1 dsRNA (45). All dsRNA was purified by CF-11 chromatography (7), and no detectable single-stranded RNA was present.

Purification of ScV particles and ScV proteins. ScV particles were purified by differential centrifugation, polyethylene glycol precipitation, and two cycles of sucrose gradient velocity sedimentation (45). The putative *cap-pol* fusion polypeptide and P1 were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 7.5% polyacrylamide gels and eluted from gel slices by diffusion into 50 mM Tris hydrochloride, pH 7.5-0.1% SDS-0.15 M NaCl-1 mM dithiothreitol-1 mM EDTA at 37°C with shaking, dialyzed against distilled water, and lyophilized.

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1	GAAAAATTTTAAATTCATATAACTCCCCATGCTAAGATTGGTTACTAAAACTCTCAAGATAAATCGTCTGATC	75
	MetLeuArgPheValThrLysAsnSerGlnAspLysSerSerAspL	
76	TATTCTCTATTTGTTCTGATCGCGAACTTTTGTGCTCATAATAGAGTTCGGACTGATTTCAGTTTGACAACT	150
	euPheSerIleCysSerAspArgGlyThrPheValAlaHisAsnArgValArgThrAspPheLysPheAspAsnL	
151	TAGTATTTAACCGAGTTTATGGTGTCTTCAAAAATTTACGTTAGTTCGGTAACCCGACAGTCTGCTTTAATGAAG	225
	euValPheAsnArgValTyrGlyValSerGlnLysPheThrLeuValGlyAsnProThrValCysPheAsnGluG	
226	GCAGTTCTTACCTAGAGGTATTGCTAAAAAGTACCTAACTTTGGATGGAGGACTTGCCATTGACAATGTCTCTCA	300
	lySerSerTyrLeuGluGlyIleAlaLysLysTyrLeuThrLeuAspGlyGlyLeuAlaIleAspAsnValLeuA	
301	ATGAGTTGAGATCCACTTGCGGTATCCCAGGTAATGCTGTTGCTCTCATGCGTATAATATTACATCTGGCGTT	375
	snGluLeuArgSerThrCysGlyIleProGlyAsnAlaValAlaSerHisAlaTyrAsnIleThrSerTrpArgT	
376	GGTATGACAATCACGTGGCACTGTTGATGAACATGTTGCGTGTACCCTTACAAGTATTGACCGAACAGGGCC	450
	rpTyrAspAsnHisValAlaLeuLeuMetAsnMetLeuArgAlaTyrHisLeuGlnValLeuThrGluGlnGlyG	
451	AATATAGCGCTGGAGATATCCCTATGTACCATGATGGACATGTCAAAATCAAGTACCAGTGACTATCGATGACA	525
	lnTyrSerAlaGlyAspIleProMetTyrHisAspGlyHisValLysIleLysLeuProValThrIleAspAspT	
526	CGGCAGGCCCAACACAATTGCTTGGCCTAGTGACAGGCTACTGATTGCTATCTGATTGGGCACAGTTTCTG	600
	hrAlaGlyProThrGlnPheAlaTrpProSerAspArgSerThrAspSerTyrProAspTrpAlaGlnPheSerG	
601	AATCATTTCATCAATCGACGTCCGTACCTAGATGTTAGGCCATTGACCGTAACGGAAGTCAATTTCTGTGCTTA	675
	luSerPheProSerIleAspValProTyrLeuAspValArgProLeuThrValThrGluValAsnPheValLeuM	
676	TGATGATGAGTAAGTGGCATAGACGTACTAAGTACGATAGACTACGAGGCACCCCACTAGCTGATAAGTTCCG	750
	etMetMetSerLysTrpHisArgArgThrAsnLeuAlaIleAspTyrGluAlaProGlnLeuAlaAspLysPheA	
751	CTTACCGCCATGCGCTTACTGTTCAAGACGCTGACGAGTGGATAGAAGCGATAGAAGTATGACAGGTTCGCGC	825
	laTyrArgHisAlaLeuThrValGlnAspAlaAspGluTrpIleGluGlyAspArgThrAspAspGlnPheArgP	
826	CCCCCTCGTCTAAAGTAAATGTTATCGGCACTTCGTAAGTACGTGAACCGTAACAGGCTGTACAATCAGTTTACA	900
	roProSerSerLysValMetLeuSerAlaLeuArgLysTyrValAsnArgAsnArgLeuTyrAsnGlnPheTyrT	
901	CTGCAGCACAACGTAGCTCAATATGATGAAACCTGTCCCTAAGTGGCTGAGGGCTACGCTTGGCTGATGC	975
	hrAlaAlaGlnLeuLeuAlaGlnIleMetMetLysProValProAsnCysAlaGluGlyTyrAlaTrpLeuMetH	
976	ATGACGCTTGGTCAATATACCAAAATTTGGGTCTATTGAGGAAGGTACCCTTTTTTGTATCAGGTGATGCAG	1050
	isAspAlaLeuValAsnIleProLysPheGlySerIleArgGlyArgTyrProPheLeuLeuSerGlyAspAlaA	
1051	CGTTGATTACAGGCTACAGCCCTAGAAGACTGGTCTGCTATCATGGCGAAACCCGAGCTGGTGTCACTTACGCGA	1125
	laLeuIleGlnAlaThrAlaLeuGluAspTrpSerAlaIleMetAlaLysProGluLeuValPheThrTyrAlaM	
1126	TGCAGGTGTCTAGTAGCGTTAAACACCGGACTATACTTACGTCGCGTTAAGAAAACAGGCTTCGGCACAACATAG	1200
	etGlnValSerValAlaLeuAlaGlnIleMetMetLysProValProAsnCysAlaGluGlyTyrAlaTrpLeuMetH	
1201	ATGACAGCTATGAAGATGGAGCGTTTTTGAACCGGAGACGTTTCGTTACGGCCGACTAGCATGTTGTACGGAC	1275
	spAspSerTyrGluAspGlyAlaPheLeuGlnProGluThrPheValGlnAlaAlaLeuAlaCysCysThrGlyG	
1276	AAGATGCGCCCCATAATGGGATGTCAGATGTGTATGTCATTATCCAGATCTTCTAGAATTGATGCTGTTACAC	1350
	lnAspAlaProLeuAsnGlyMetSerAspValTyrValThrTyrProAspLeuLeuGluPheAspAlaValThrG	
1351	AAGTACCCATCACGGTCATTGAGCCCGCTGGCTATAACATTGTTGATGATCATTAGTGTTGTGGGTGTAACCTG	1425
	lnValProIleThrValIleGluProAlaGlyTyrAsnIleValAspAspHisLeuValValValGlyValProV	
1426	TGGCATGTTACCATACATGATATTTCCAGTAGCTGCGTTTGATACTGCAAATCCTTACTGTGGGAATTTGTCA	1500
	alAlaCysSerProTyrMetIlePheProValAlaAlaPheAspThrAlaAsnProTyrCysGlyAsnPheValI	
1501	TTAAGGCTGCTAACAAAGTATCTCCGTAAGGGTGCCGTGTATGATAAACTCGAAGCATGGAAGTTGGCTGGGCAC	1575
	leLysAlaAlaAsnLysTyrLeuArgLysGlyAlaValTyrAspLysLeuGluAlaTrpLysLeuAlaTrpAlaL	
1576	TGAGGTAGCCCGGTATGACACTCACTTCAAAGTGCATGGCGATACACAGGCTTAAGTTCATGCTGACA	1650
	euArgValAlaGlyTyrAspThrHisPheLysValHisGlyAspThrHisGlyLeuThrLysPheAlaAspA	
1651	ACAGTGACACATGGACACACATACCTGAATTTGTCACTGACGGTGACGTGATGGAAGTATTCGTTACTGCCATCG	1725
	snSerAspThrTrpThrHisIleProGluPheValThrAspGlyAspValMetGluValPheValThrAlaIleG	
1726	AACGCAGAGCTAGACATTTTCGTTGAACACTACCTAGACTGAATTCACCAGCATTCTTCAGATCTGTAGAAGTCAGCA	1800
	luArgArgAlaArgHisPheValGluLeuProArgLeuAsnSerProAlaPhePheArgSerValGluValSerT	
1801	CCACTATATATGATACTCATGTGCGAGCTGGTGGCATTGATGATATCATGCTAGACGAATCAATCTTGATTATG	1875
	hrThrIleTyrAspThrHisValGlnAlaGlyAlaHisSerValTyrHisAlaArgArgIleAsnLeuAspTyrV	
1876	TTAAGCCTGTTTCGACCGGCACTTCAAGGTGATCAATGCGGGCGAACTTAAGAACTACTGGGGTAGTGTGCGTCGTA	1950
	alLysProValSerThrGlyIleGlnValIleAsnAlaGlyGluLeuLysAsnTyrTrpGlySerValArgArgT CysAlaSerTy	
1951	CTCAGCAGGGTTTAGGAGTGGTAGGTCTTACGATGCCAGCTGTAATGCCTACCGGAGAACCTACAGCTGGCGCTG	2025
	hrGlnGlnGlyLeuGlyValValGlyLeuThrMetProAlaValMetProThrGlyGluProThrAlaGlyAlaA rSerAlaGlyPheArgSerGlyArgSerTyrAspAlaSerCysAsnAlaTyrArgArgThrTyrSerTrpArgCy	
2026	CCCACGAAGAGTTGATAGAACAGCGGACAATGTTTTAGTAGAGTAAACGTAATCGAACCTCACACGGACCCCG	2100
	laHisGluGluLeuIleGluGlnAlaAspAsnValLeuValGlu sProArgArgValAspArgThrGlyGlyGlnCysPheSerArgValAsnValIleGluProSerHisGlyProAr	
2101	CCCTACAAGGTACATACTGCAGGAACAGGTACGTACCTGCGTGGATTAGATTGAGGAACAGAGTACAAGCTGT	2175
	gProThrArgTyrIleLeuGlnGluProGlyThrTyrProAlaTrpIleArgPheArgAsnArgValGlnAlaVa	
2176	ATCGCGTCAGAAAGCCACTCACTTCTTGTGTTGACATCGTACCTGCGCGAGTAATTAGTGATTTTACTACGTCTGA	2250
	lSerArgGlnLysAlaThrHisPheLeuPheAspIleValProAlaAlaValIleSerAspPheThrThrSerAs	
2251	CACGTCTTCGTTTGCATACAAATCGCACACCTACGCTGCAATGTAACAGCATTGAGGTTCACTGACACTTATGC	2325
	pThrSerSerPheAlaTyrLysSerHisThrTyrAlaAlaAsnValThrAlaLeuArgPheSerAspThrTyrAl	

FIG. 1. Sequence of L1. The sequence of the plus-strand cDNA is given. The amino acid sequences predicted by the two large open reading frames are shown. Where there overlap (1940 to 2069), the upper line gives the *cap* sequence and the lower line gives the *pol* sequence. The sequence is available as EMBL accession number X13426.

2326	CTTGACGTACAGACTGATACCAATATGACAATTTTAAGCCACGCGCGCTCGCCAGGCTTCTGCGACGTACTC aLeuTyrValGlnThrAspThrAsnMetThrIleLeuSerProAlaAlaArgArgGlnAlaSerAlaThrTyrSe	2400
2401	ACAGGTGGCAGGGTTTTGTTATAACACACCTACCGTTATGGATTTCGCTAGCGAATATCTTGGACGTAGACCGCAA rGlnValAlaGlyPheCysTyrAsnThrProThrValMetAspSerLeuAlaAsnIleLeuAspValAspArgAs	2475
2476	TATACGACCCAAACACTTCAAGGGTTTACGGCTATACACCAGCTCTAAGGTCAGTCTCAACATCATACTCACTT nIleArgProLysHisPheLysGlyLeuArgLeuTyrThrSerSerLysValThrAlaGlnHisHisThrHisLe	2550
2551	GCGGCCAGACGAGCTAGTGGAGCGGCCCAAGGCTCGCCTAGACGTAATACTACCTAATGTGTGTAGTTGA uArgProAspGluLeuValGluAlaAlaAlaLysValSerProArgArgLysTyrTyrLeuMetCysValValGl	2625
2626	GCTGCTCGGCAACTTACAAGTAGATCTTGAAGCAGCAGTAGCTACTATTCTAGCATATGCTCTGACACTAAGTGA uLeuLeuAlaAsnLeuGlnValAspLeuGluAlaAlaValAlaThrIleLeuAlaTyrValLeuThrLeuSerGl	2700
2701	AAAATTTGTACCAATTTTCTTGGATTCTAGAGCAATATGGGTCGGTGAGCCTGGGCTGATGCTCTGACTGCACG uLysPheValProIlePheLeuAspSerArgAlaIleTrpValGlyGluProGlyProAspAlaLeuThrAlaAr	2775
2776	TCTCAAGGCCAGTAGTGGGCAGATCAAGAGCATACACGGCTGATTACGAACCACTCACTGAATATTCGAGTT gLeuLysAlaSerSerGlyGlnIleLysSerIleHisThrAlaAspTyrGluProLeuThrGluLeuPheGluLe	2850
2851	AGCAGTATTGATGAACCGAGGTGTTGCCATGTCTCTTGGCAAGCTGAAAAGGATCATCGCTTGAATCCCGACGT uAlaValLeuMetAsnArgGlyValGlyHisValSerTrpGlnAlaGluLysAspHisArgLeuAsnProAspVa	2925
2926	GGCTGTAGTTGATCAAGCACGGCTATATTCGTGTGTGCGGACATGTTTCAAGGATCAAGCAGACGTATAAATA lAlaValValSerPheAlaArgLeuTyrSerCysValArgAspMetPheGluGlySerLysGlnThrTyrLysTy	3000
3001	TCCCTTTATGACGTGGGATGACTACACTGCAACAGATGGGATGGGTTCCAGGTGGCAGTGTCCACTCTCAATA rProPheMetThrTrpAspAspTyrThrAlaAsnArgTrpGluTrpValProGlyGlySerValHisSerGlnTy	3075
3076	CGAAGAAGACAACGATTATATCTATCCTGGTCAGTATACTAGGAACAAGTTCATAACTGTTAACAAAAATGCCAA rGluGluAspAsnAspTyrIleTyrProGlyGlnTyrThrArgAsnLysPheIleThrValAsnLysMetProLy	3150
3151	ACACAAAATATCTAGAATGATAGCATCACCGCTGAGGTACGAGCTTGGACGTCGACGAAGTACGAATGGGGCAA sHisLysIleSerArgMetIleAlaSerProGluAlaTrpThrSerThrLysTyrGluTrpGlyLy	3225
3226	GCAACGTGCTATCTACGGGACGGATCTACGAAGTACACTGATACTTAATTTGCAATGTTCAGGTGCGAGGATGT sGlnArgAlaIleTyrGlyThrAspLeuArgSerThrLeuIleThrAsnPheAlaMetPheArgCysGluAspVa	3300
3301	TCTCACTCACAAGTTCCCGTAGGCGACCGAGGCGAGGCGACGAAAGGTGCACAAACGGGTGAACATGATGCTGGA lLeuThrHisLysPheProValGlyAspGlnAlaGluAlaAlaLysValHisLysArgValAsnMetMetLeuAs	3375
3376	CGGTGCCTCTAGTTTCTGCTTCGATTATGATGACTTCAATTCTCAGCATTCGATAGCTAGTATGTATACGGTTTT pGlyAlaSerPheCysPheAspTyrAspAspPheAsnSerGlnHisSerIleAlaSerMetTyrThrValLe	3450
3451	GTGCGTTTTCAGGGACACATTTAGTCGCAACATGTCTGATGAACAAGCAGAGGCGATGAAGTGGGTGTGTGAGTC uCysAlaPheArgAspThrPheSerArgAsnMetSerAspGluGlnAlaGluAlaMetAsnTrpValCysGluSe	3525
3526	CGTCAGACACATGTGGGTACTAGATCTGATACCAAGGAGTGGTACAGACTACAAGGTACATTACTGTCAGGATG rValArgHisMetTrpValLeuAspProAspThrLysGluTrpTyrArgLeuGlnGlyThrLeuLeuSerGlyTr	3600
3601	GCGGTTAACCACATTTATGAATACTGTGCTAACTGGGCGTATATGAAATTAGCTGGCGTATTTGATCTGGATGA pGlyLeuThrPheMetAsnThrValLeuAsnTrpAlaTyrMetLysLeuAlaGlyValPheAspLeuAspAs	3675
3676	CGTTCAAGACTCGGTACACAACGGGTGATGATGTTATGATTAGTCTCAACCGGTGAGCACAGCAGTGAGAATAAT pValGlnAspSerValHisAsnGlyAspAspValMetIleSerLeuAsnArgValSerThrAlaValArgIleMe	3750
3751	GGACGCTATGCACCGGATAAATGCGCGAGCAGACCGCGGAAGTGTAACCTGTTTTCGATAAGTGAATTTCTGAG tAspAlaMetHisArgIleAsnAlaArgAlaGlnProAlaLysCysAsnLeuPheSerIleSerGluPheLeuAr	3825
3826	GGTAGAACACGGTATGAGCGGAGGCGATGGTCTTGGGGCTCAGTACTTAAGTAGGTCTTGTGCTACTCTTGTA gValGluHisGlyMetSerGlyGlyAspGlyLeuGlyAlaGlnTyrLeuSerArgSerCysAlaThrLeuValHi	3900
3901	CAGTAGGATTGAGTCTAACGAACCACTGTCACTAGTACGAGTTATGGAAGCAGACCAGGCTAGATTGCGCGACCT sSerArgIleGluSerAsnGluProLeuSerValValArgValMetGluAlaAspGlnAlaArgLeuArgAspLe	3975
3976	GGCAACAGAACGCGGGTACAATCTGCGGTAAACAGCGATAAAGAACAACCTCGACAAACGTGTCCTAAGATATT uAlaAsnArgThrArgValGlnSerAlaValThrAlaIleLysGluGlnLeuAspLysArgValThrLysIlePh	4050
4051	CGGAGTTGGTGATGACGTTGTGCGCGACATACACAGCTCACAGGTTGTGTGGCGGTATCTCGACTGATACCTG eGlyValGlyAspValValArgAspIleHisThrAlaHisArgValCysGlyGlyIleSerThrAspThrTr	4125
4126	GGCACCAGTTGAACTAAGATAATAACAGACAATGAAGCATATGAAATACCATACGAAATAGATGATCCATCATT pAlaProValGluThrLysIleIleThrAspAsnGluAlaTyrGluIleProTyrGluIleAspAspProSerPh	4200
4201	TTGGCCAGGGTAAATGATTATGCTTATAAAGTCTGGAATAATTCGGAGAACGACTCGAATTTAATAAAATTAA eTrpProGlyValAsnAspTyrAlaTyrLysValTrpLysAsnPheGlyGluArgLeuGluPheAsnLysIleLy	4275
4276	AGATGCCGTAGCTAGAGGGAGTAGGAGCACTATAGCTCTGAAACGTAAGGCTAGGATAACATCTGAGAAGAATGA sAspAlaValAlaArgGlySerArgSerThrIleAlaLeuLysArgLysAlaArgIleThrSerGluLysAsnGl	4350
4351	ATTCGTAACAAGTCGGAATGGGAAAGACAATGTACAAAGCCTATAAGGGTTTGGCAGTCTCATACTATGCTAA uPheAlaAsnLysSerGluTrpGluArgThrMetTyrLysAlaTyrLysGlyLeuAlaValSerTyrTyrAlaAs	4425
4426	CCTGAGCAATTCATGAGTATACCACCAATGGCGAACATTGAATTTGGGCAGGCTAGATATGCTATGCAAGCAGC nLeuSerLysPheMetSerIleProProMetAlaAsnIleGluPheGlyGlnAlaArgTyrAlaMetGlnAlaAl	4500
4501	CCTTGATAGTTCTGATCCACTCCGGGCATTACAGGTCATACTGTAGTTGCCAAAAAGATAATGGGAATTACCCAT aLeuAspSerSerAspProLeuArgAlaLeuGlnValIleLeu	4575
4576	ATGCA	4580

FIG. 1—Continued.

Antibodies, ELISA and Western blots. A synthetic peptide, derived from the putative RDRP sequence, was made by the SUNY/AB Center for Applied Molecular Biology and Immunology and used to raise antibodies in rabbits. This was ScV2, EDNDYIYPGQYTRNK, residues 381 to 395. The peptide was cross-linked to keyhole limpet hemocyanin at an average of 7 mol of peptide per mol of keyhole limpet hemocyanin by treatment with 0.1% glyceraldehyde at room temperature for 1 h, and 200 to 300 μ g in Freund complete adjuvant was injected into rabbits. Booster injections in Freund incomplete adjuvant were administered at 2- to 3-week intervals five times. Preimmune serum was purified by passage over protein A-Sepharose (24). The immune serum was purified on Nalgene-activated affinity columns complexed with ScV2 peptide according to the instructions of the manufacturer. The anti-P1 antibody was made by injecting rabbits with SDS-PAGE-purified P1 (K. A. Bostian, personal communication). Enzyme-linked immunosorbent assays (ELISA; 17, 21) were performed with plates coated with purified ScV particles or with the synthetic peptide. Detection was by protein A-horseradish peroxidase (15). Assays were run at room temperature for 20 min, and the assayed material was diluted 20-fold before being read at 490 m μ . Preimmune antibody was prepared from the same rabbits used to produce the antipeptide antibody prior to immunization. Western blots were performed as previously described (31) by using purified immunoglobulin G (preimmune) or affinity-purified antibody (anti-ScV2) and detection with iodinated protein A. Antibodies at the same concentration (5.4 mg/ml of protein) were diluted 1/25.

RESULTS

Sequence of L1. The sequence of L1 was derived from a total of 34 independently isolated, overlapping cDNA clones, a minimum of 12 of which included the entire sequence, except the first 71 b. These clones, depending on how they were derived, varied from 24 to 1,135 b in length. The sequence was obtained on both strands, except for the first 71 b and b 314 to 447, by appropriate subcloning or by synthesis of oligonucleotide primers. Portions of the sequence have been reported previously (10, 44). Regions at both ends were completed by direct RNA sequencing. The first 71 b agree with the end sequence previously reported (50), except for a U-for-C change at position 26. Many individual sequencing runs have been repeated several times, especially in the two short regions sequenced only on one strand. The sequence and its open reading frames are shown in Fig. 1.

The previously observed inverted repeat (10) is a cloning artifact; two independent cDNA clones encompassing the region in question (around b 2174) diverge from this reported sequence at b 2174 and have identical sequences in the overlapping region. The clone with this inverted repeat appears to have been derived by dissociation of reverse transcriptase from the L1 RNA plus-strand template at b 2174 and reassociation with the nascent DNA strand at b 2509, where a 5-b inverted repeat of the sequence at 2174 occurs, followed by synthesis back to b 2778.

The total length of 4,580 base pairs (bp) is closer to the size estimated from 5'-end analysis (4.5 kbp [recalculated from data in reference 8]) than to sizes estimated by electron microscopy (4.8 kbp [11]) or equilibrium gradient centrifugation (5.2 kbp [29]).

Genome organization of L1. There are two large open reading frames on the L1 plus strand and none on the minus

FIG. 2. Genome organization of ScV-L1. The capsid polypeptide (cap) and RDRP (pol) open reading frames and the predicted sizes of the encoded polypeptides are shown. kd, Kilodaltons.

strand (Fig. 2). We have designated the first large open reading frame, from b 30 to 2072, *cap*, since it encodes the major capsid polypeptide P1 (see Discussion). The predicted size of P1 is 76 kDa, on the low end of sizes estimated by SDS-PAGE.

The second open reading frame, *pol*, from b 1940 to 4543, overlaps with *cap* for 129 coding bases. It has no methionine until b 2351. The predicted polypeptide, a portion of which we have previously analyzed and identified as a putative RDRP (44), shares extensive homology with all the known or suspected plus-strand viral RDRPs (34), as shown in Fig. 3 and 4. The homology extends over 223 amino acids in the first region and 102 amino acids in the second, separated by 110 amino acids without homology to viral RDRPs. All but 2 of the 44 highly conserved residues are preserved in the ScV RDRP sequence. The one conserved region also present in reverse transcriptases and Qbeta replicase (the GDD region) is duplicated in the ScV RDRP on either side of the 110-amino-acid insertion.

A previously reported sequence (44; EMBL accession number X07770), obtained from a separately isolated cDNA clone containing 1,135 b of the 3' end of L1, has no termination codon. Missing from this sequence are the G and T, b 4543 and 4544, of the sequence reported here; the T (U in the RNA) is the first base of the termination codon UAG. The region of the sequence in the present report, obtained from another cDNA clone and from direct sequencing of the RNA, agrees with previously published data (19, 50). The last base (4580) is added posttranscriptionally (7).

We examined, in two ways, the question of whether this second open reading frame is expressed: by searching for a polypeptide with predicted epitopes in viral particles and by *in vitro* translation. By analogy with retrovirus *gag-pol* fusion proteins resulting from frameshift translation of overlapping reading frames (5, 32-35, 56), we suspected that such a protein would result from translation of the L1 transcript. Its predicted size would be about 171 kDa (Fig. 2).

Presence of ScV RDRP in viral particles. Only two polypeptides have been well documented in ScV-L1 particles: P1 and a much larger polypeptide of 170 to 180 kDa (45, 48). This larger polypeptide is already known to possess epitopes in common with P1 (45). We synthesized a peptide derived from the sequence of the putative RDRP (see Materials and Methods). Polyclonal antibodies raised in rabbits to this peptide recognized the peptide (and the conjugating protein, keyhole limpet hemocyanin) in ELISA (not shown). Affinity-purified antibody to ScV2 continued to have a high titer against ScV2, with an ELISA reaction still detectable after

SNBV	2186	RFVMDMKRDV-KVTPGTK-HTEE-----RPK-VQV	2212
ScV	377	SQYEDNDYIYPGQ-YTRNKFITVNKMPKHKISRM	410
Polio	156	TYVKDELRSKTKV-----EQGK-SRL	175
		++ ++ + ++	***
SNBV	2213	IQAAEPLATAYLCG-----IH	2228
ScV	411	IA-SPEVRAWSTKYEWGKQRTAIYGTDLRSTLI	444
Polio	176	IEASSLNDVAMRM-----AF	191
		+ + + + +	++
SNBV	2229	RELVR-RLTAVLLPNIHTLFDMSAE--DFDAITAE	2260
ScV	445	TNFAMFRCEVDLTHEVARVGDQ-AEAAKVHKRVNM	478
Polio	192	GNLYA-AFHKNFGVITGSAGCDFD-LFWSKIPVL	224
		++++ ++ ++ + + + + + + + +	+
SNBV	2261	HFQKQDPVLETDIASFDKS-QDDA-MALTGLMILE	2293
ScV	479	MLDGASSFC-FDYDDF-NSQHSIASMY-TVLCAPR	510
Polio	225	MEEKL--FA-FDYTYGDASLS-PA-W--FEAL--K	250
		+++ + ++ +++ + + + + + + + + +	***
SNBV	2294	D-LGVDFLLDLIECA--FGEISSTHL---P-TGT	2321
ScV	511	DTF-SR-NMSDEQAEMNVVCSVRHMVLDPTKE	544
Polio	251	M-VLEKIGF-GDRVDYIDYLNH-SHHL---YCKNK	278
		+ + + + + + + + + + + + + + + +	+
SNBV	2322	RFKFGAMMKSGLFLLFVNTVLNVVIASR-VLE--	2353
ScV	545	WYRLQGTLLSGWRLTFMNTVLNWAYM-K-LAG--	575
Polio	279	TYCVKGGMPGSGSGTSIFNSMINNLI-RTLLKLT	312
		+++ + + + + + + + + + + + + + + + +	***
SNBV	2354	---ERLKTSRCAAFIGDDNIIH	2372
ScV	576	--VFDLDDVQDSVHNGDDVMIS	595
Polio	313	YKGIDLDHL-KMIAYGDDVIAS	333
		++++ + + + + + + + +	***

FIG. 3. Homologous regions in RDRPs: amino-terminal region. Symbols, +, functional homologies between the ScV RDRP and either Sindbis virus (SNBV) or poliovirus (polio) polymerases; *, residues highly conserved among the eight RDRPs excluding ScV. The alignment of the poliovirus and Sindbis virus RDRPs is from Kamer and Argos (36). The ScV RDRP is aligned with these to maximize homology. For the ScV polymerase, amino acid numbers are given starting at the first possible residue in the second open reading frame (starting at b 1940 of the L1 plus strand). For Sindbis virus, they are given starting at the amino terminus of the Sindbis virus polyprotein. The region shown is within nonstructural protein 4 of SNBV.

10,000-fold dilution, but no longer recognized keyhole limpet hemocyanin. Control antibodies from preimmune rabbits gave no reaction, and polyclonal antibody to P1 (made against P1 purified by SDS-PAGE) also failed to recognize the synthetic peptide.

Western blots with these antibodies are shown in Fig. 5. Approximately 1 μ g of P1 and a smaller amount of the putative *cap-pol* fusion protein purified by a single cycle of SDS-PAGE were loaded on neighboring lanes of an 8% polyacrylamide gel, and several Western transfers were

SNBV	2362	AAFIGDDNIIHGVSDKEMAE-RCATWLNMEVKII	2395
ScV	705	IFGVGDDVVRDIHTAHRVCGGISTDTWAPVETKII	739
Polio	323	MIAVGDDVIA-----SYP--HEVDASLL	343
		++ +++++ + + ++ ++ + + + + +	***
SNBV	2396	-DAVIGERPPY-----FCGGF--ILQ-----	2413
ScV	740	TDNEAYE-IPYEIDDPSPFWGVNDYAYKVWKNFGE	773
Polio	344	-AQSGKDYGLT-----MTPAD-KSATF-----	363
		++ + + + + + + + + + + + + + +	+
SNBV	2414	-----DSVT-STACRVADPLKRLFKL-GKPLP	2438
ScV	774	RLEFNKIKDAVARGSRSTIA--LKRKARITSEKNE	806
Polio	364	-----ETVTW-----ENVTV-FLKRFRA-DEKYP	385
		+ + + + + + + + + + + + + + + +	***

FIG. 4. Homologous regions in RDRPs: carboxy-terminal region. Symbols, abbreviations, and alignments are as described in the legend to Fig. 3.

FIG. 5. Western blots of P1 and the putative *cap-pol* fusion protein purified from viral particles. (A) Reaction with anti-ScV2 affinity-purified antibody. (B) Reaction with anti-P1 antibody. The positions of the P1 monomer, P1 trimer, possible pentamer, and putative *cap-pol* fusion protein, as well as the marker proteins (sizes in kilodaltons) are shown. Lanes: 1, purified *cap-pol* fusion protein (about 0.1 μ g); 2, purified P1 (about 1 μ g). These were transfers from the same 7.5% polyacrylamide gel.

performed. Anti-ScV2 purified on affinity columns of ScV2 peptide recognized the putative fusion protein but not P1. Anti-P1 recognized both P1 and the putative *cap-pol* fusion protein. The preimmune immunoglobulin G from the rabbits producing anti-ScV2 recognized none of the proteins on the Western transfer (not shown). These results are consistent with the predicted translational frameshift. In addition, purified P1 aggregated in vitro, producing products that migrated with the molecular weights of dimers, trimers, and possible pentamers. Some degradation products of P1 and of the *cap-pol* fusion protein are also visible.

Synthesis of the *cap-pol* fusion product in vitro. We next examined whether the predicted translational frameshift could occur in vitro. A mammalian system was used to translate purified, denatured L1 dsRNA. No single-stranded viral or host RNAs were present in the reactions. As before (6, 30, 45), the major product was P1, although many smaller products, presumably due to premature termination, also occurred (Fig. 6). Using dsRNA carefully prepared to prevent nicking, in vitro translation produced a large polypeptide of about 170 to 180 kDa as well as a series of smaller products that may have been due to premature translation termination or to low levels of RNA or protein degradation. Translation of undenatured L1 dsRNA (a test for contaminating single-stranded RNAs) gave no in vitro products (not shown). Since the only way a 170- to 180-kDa product could be synthesized in vitro on the L1 dsRNA template is by translational frameshift within the overlap between *cap* and *pol*, this must be a *cap-pol* fusion product. The amount of the *cap-pol* product was about 5.9% that of P1 on a mass basis. This is similar to what is observed by translational frameshift with retrovirus RNA (33). It is also similar to the relative amount of the 170- to 180-kDa polypeptide in ScV virions (3.6%) estimated by scanning the gel shown in Fig. 6. This corresponds to 0.97 copies of a 171-kDa polypeptide in particles with 60 copies of P1 (45). In this gel, P1 migrated with a molecular size of 82 kDa, and the putative RDRP migrated with a molecular size of 170 kDa. We conclude that the mechanism of translation of the RDRP is as a fusion product with P1.

FIG. 6. In vitro translation of L1. (a) Polyacrylamide gel (8%) of ScV viral proteins stained with Coomassie brilliant blue. (b) Autoradiograph of an 8% polyacrylamide gel of the [35 S]methionine-labeled in vitro translation products of denatured L1 in a mammalian system (see Materials and Methods). Small translation products may be due to premature translation termination or to RNA or protein degradation. Marker polypeptides were vesicular stomatitis virus M (29 kDa [kd]), vesicular stomatitis virus N (50 kDa), albumin (68 kDa), phosphorylase *b* (97 kDa), beta-galactosidase (116 kDa), and vesicular stomatitis virus L (190 kDa).

DISCUSSION

With one exception (18, 39), all of the viral dsRNAs sequenced to date show one open reading frame. In contrast, L1 has two large open reading frames on the plus strand and none on the minus strand. The plus strand was identified by in vitro translation and sequence analysis of viral transcript (4, 6). As shown in Fig. 2, the first open reading frame begins with a methionine at b 30 and ends at 2069, or at 2072 if the UAA termination codon is included. This open reading frame is undoubtedly the P1 gene, which we have designated *cap*, since (i) L1 is known to encode the major capsid polypeptide (30, 45), (ii) yeast ribosomes initiate at the first AUG (1), (iii) the size of P1 is consistent with the coding capacity of this open reading frame, and (iv) there is no other large open reading frame initiated by an AUG. In vitro translation of denatured L1 or its plus strand results primarily in synthesis of products derived from P1, the largest of which was estimated as 88 kDa, and coelectrophoreses with P1 (6, 30, 45). The predicted size of the P1 peptide from the *cap* sequence is 76 kDa, at the low end of the range of reported sizes for P1 (42). The predicted sequence has some similarity, in its central 250 amino acids, to the picornavirus vp3 (J. A. Bruenn, M. E. Diamond, and J. J. Dowhanick, submitted for publication).

It appears that the dsRNA viruses have RDRPs related to those of their single-stranded RNA cousins. At least one other dsRNA viral polymerase also has some sequence similarity to the RDRPs of plus-strand RNA viruses (25). It is interesting that the putative ScV RDRP has two Glu-Asp-Asp (GDD) consensus sequences, since this region is implicated in some process common to all RDRPs and to reverse transcriptases (36). Since the ScV polymerase, unlike the RDRPs of plus-strand RNA viruses, uses a dsRNA template in its first enzymatic reaction (transcription), the

two GDD sequences might both function: one in transcription and one in replication (44).

The genomic organization of the *S. cerevisiae* L1 viral dsRNA is somewhat similar to that of the retroviruses. On the viral plus strand, the viral capsid polypeptide gene (*cap*) is at the 5' end, followed in an overlapping reading frame by a polymerase gene (*pol*). Translation of the polymerase appears to occur by translational frameshift, in this case by a -1 frameshift, as in the retroviruses and at least one other RNA virus (5, 32–35, 38, 56). The yeast TY elements have a $+1$ frameshift responsible for synthesis of the TYA-TYB fusion polypeptide (12). The *gag-pol* overlaps vary from 13 to 205 b (33) in comparison with the 38-b overlap of TYA and TYB and the 129-b overlap of ScV *cap* and *pol*. The TYA-TYB frameshift does not require any homopolymeric runs (56), but the retrovirus frameshift often takes place by ribosomal slippage at the sequence UUUA or UUUUA, resulting in pairing of a leucine tRNA with the phenylalanine codon UUU (33). The sequence UUUUA does occur in the *cap-pol* overlap, at b 2059 to 2063, and the result would be the same: placing a leucine tRNA at the UUU codon. In some cases, frameshifting may require more than the immediate sequence around the frameshift site, including a stem and loop structure immediately following the frameshift site (32, 34). There is a highly stable (-27 kcal/mol [112.97 kJ/mol]) stem and loop structure possible at b 1984 to 2022, which is preceded by a UUUA sequence at 1961 to 1964. Since yeast ribosomes accomplish the HIV frameshift in vivo with about the same efficiency as mammalian ribosomes, requiring only 16 bases containing a UUUUA sequence, and without any secondary structure cues (56), it is unclear what the necessary sequences in the *cap-pol* overlap are.

We have not shown that expression in vivo proceeds in this manner, but we have reason to think that it does. The putative RDRP could be translated on an unmodified L1 plus strand by frameshifting or on an L1 plus strand modified by splicing, posttranscriptional editing, or cleavage. Splicing occurs in influenza virus, which is thought to be transcribed in the nucleus (37). This possibility seems remote in ScV, since the particles are known to be cytoplasmic and transcription takes place in intact particles. There are no DNA copies of the ScV genome (26). Posttranscriptional editing occurs in some minus-strand RNA viruses (51). We cannot rule out this possibility in ScV in vivo, but clearly translation in vitro of unprocessed plus strands produces the suspected fusion protein. Cleavage of the L1 plus strand could create a subgenomic RDRP mRNA. The in vivo L1 mRNA is thought to be a full-length transcript (27), although there is a report of a 5', but not a 3', subgenomic fragment (2). Only the full-sized L mRNA is synthesized by particles in vitro (6; M. E. Nemeroff and J. A. Bruenn, unpublished data). The existence of the apparent fusion protein in viral particles also militates against the possibility of cleavage to create a subgenomic mRNA.

The predicted RDRP is found in ScV particles, in the form of a fusion protein of 171 kDa. We have found no evidence for posttranslational cleavage of the *cap-pol* fusion product. ScV has a very condensed genome, in comparison with the reoviruses. Not only are all but 67 of the bases of L1 coding, but they encode two coding sequences in overlapping reading frames. In reovirus, the major capsid polypeptide, which accounts for only 35% of the total protein mass, is encoded by one RNA segment (49, 54) and the polymerase by another (14). The simplicity of the ScV genome and virion promises to make the ScV system useful for understanding the enzy-

matic and structural characteristics of its proteins. These may have general application to other dsRNA viruses.

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ADDENDUM

In work published after submission of this report, three peptides derived from P1 by proteolytic digestion were shown to have sequences that correspond to those predicted by our cDNA sequence of *cap* (13). Immunological results published after submission of this report confirm that there are two overlapping reading frames in L1 (23).

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